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Chlorsulfuron: agronomic evaluation and physiological investigation

by

Hendrik D. Bestman

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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Date.....April 22, 1982.....

Abstract

The new herbicide chlorsulfuron was evaluated for the control of Tartary buckwheat in cereal crops in north-central Alberta. Preemergence and postplanting-incorporated treatments of up to 80 g/ha did not provide an acceptable level of Tartary buckwheat control. The efficacy of postemergence chlorsulfuron applications for the control of Tartary buckwheat was dependent upon the growth stage at the time of spraying and upon the rate of application. This efficacy was rather inconsistent. In 1980, 10 or 40 g/ha was sufficient to control Tartary buckwheat when sprayed at the 1- or 4 to 6-leaf stage respectively, while in 1981, up to 60 g/ha provided only limited control, regardless of the leaf stage at the time of spraying.

Controlled environment studies revealed that the susceptibility of Tartary buckwheat to a single-droplet application of chlorsulfuron decreased as the leaf stage at the time of application advanced from one to four, regardless of chlorsulfuron dosage. In Tartary buckwheat, chlorsulfuron caused a temporary inhibition of growth, a shriveling of the stem below the cotyledonary node, and an inhibition of stem elongation. Although the severity of chlorsulfuron-induced symptoms in Tartary buckwheat was increased by the addition of the adjuvant Cittowet Plus, in the field no significant increase in chlorsulfuron efficacy in terms of biomass at harvest time could be observed.

Chlorsulfuron at rates as low as 5 or 30 g/ha is an effective herbicide for the control of hempnettle or cleavers, respectively.

When applied in a tank mix, chlorsulfuron up to 80 g/ha did not significantly reduce the efficacy of the postemergence wild oat herbicides diclofop methyl, flamprop methyl, difenzoquat, and barban.

In the field, chlorsulfuron up to 80 g/ha did not injure wheat or barley.

The use of chlorsulfuron is not recommended if rapeseed is to be grown the following year. A two-year interval between the time of a 50 to 150 g/ha application and the seeding of rapeseed was required for the chlorsulfuron residues to dissipate below a level inhibitory to the growth of rapeseed. A corn root bioassay revealed that 18 ppb chlorsulfuron in the soil in the fall was sufficient to cause a significant inhibition of the growth of rapeseed the following year.

Chlorsulfuron concentrations as low as 2 ppb significantly reduced the root growth rate of corn seedlings. It is postulated that inhibition of cell division is the predominant effect.

In studies with ^{14}C-chlorsulfuron and Tartary buckwheat (2-leaf stage), only 81.6% of the applied activity could be recovered 120 hr after treatment; 42% was recovered in the leaf wipes, 37% from the tissue of the treated leaf, and 2.6% from the remainder of the plant. No significant

chlorsulfuron metabolism could be detected.

Mutilation studies indicated that, in Tartary buckwheat, a chlorsulfuron-treated leaf had to remain connected to the untreated remainder of the plant for only 2 hours, in order to induce typical chlorsulfuron symptoms in that plant.

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1. INTRODUCTION

The use of herbicides for the control of weeds has become a widely accepted practice in Canadian agriculture. In the three prairie provinces alone the estimated use of herbicides has increased from 2,238,000 kg in 1958 to 14,233,000 kg in 1978 (49). Although the registration of new herbicides has become a costly and time-consuming procedure, several new chemical compounds are currently being tested in order to become registered for commercial sale in Canada. One of these is chlorsulfuron.

The first objective of this study was to evaluate the performance of chlorsulfuron for the control of such broadleaved weeds as Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.], hempnettle (*Galeopsis tetrahit* L.), and cleavers (*Galium aparine* L.) in cereal crops and on fallow in the north-central region of Alberta. The second objective was to assess chlorsulfuron for carryover in the soil and its possible effect on rapeseed (*Brassica campestris*). The third objective was to examine the influence of chlorsulfuron on (i) the shoot growth of Tartary buckwheat plants after foliar application, and (ii) the root growth of corn (*Zea mays* L.) seedlings growing in solutions of this herbicide.

In order to achieve these three objectives, both field and controlled environment experiments were conducted. The field experiments were specifically designed (i) to obtain information regarding the rate of chlorsulfuron required at

a particular leaf stage of the broadleaved weeds in order to achieve an acceptable level of control, (ii) to study the effect of the adjuvant Cittowet Plus on the efficacy of chlorsulfuron, (iii) to assess the tolerance of cereal crops to this new herbicide, (iv) to explore the possibility of tank mixing chlorsulfuron with postemergence wild oat herbicides, and (v) to determine the effect of chlorsulfuron carryover in the soil on rapeseed. The controlled environment experiments were designed (i) to provide additional information regarding the required rates, the interaction with wild oat herbicides, and the tolerance of cereals to high rates of chlorsulfuron, (ii) to observe the influence of chlorsulfuron on the growth of Tartary buckwheat, (iii) to determine the concentration of chlorsulfuron in soil samples by means of a bioassay, (iv) to study the effect of chlorsulfuron on the root growth rate of corn seedlings, and (v) to quantify the absorption, translocation, and metabolism of chlorsulfuron by Tartary buckwheat, using both ^{14}C-labelled and non-labelled chlorsulfuron.

The selection of Tartary buckwheat as the main experimental weed species was based on its regular growth habit, the ease of establishing a rather uniform stand both in the field and in the controlled environments, and the availability of sufficient seed to accomplish this study. Experiments conducted in fields with a natural infestation of hempnettle and cleavers were included to broaden the

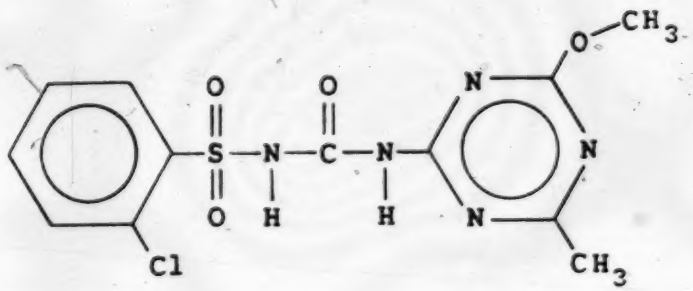
scope of the evaluation. The importance of rapeseed as a cash corp in north-central Alberta warranted a study on the effect of chlorsulfuron carryover in the soil on this crop. Because of the high sensitivity of their roots to low concentrations of chlorsulfuron, corn seedlings were particularly well suited for the bioassay.

2. LITERATURE REVIEW

2.1 Chlorsulfuron

2.1.1 Technical Information

Chlorsulfuron[1] is a new selective herbicide for weed control in cereal crops. It can be applied in both preemergence and postemergence treatments, controlling a variety of broadleaf weeds and certain grasses (61,62). Since 1979, this herbicide has been tested on a world-wide scale under the code name DPX-4189 (6). The manufacturer, E.I. DuPont de Nemours & Co. Inc., anticipates the licensing of chlorsulfuron in Canada for commercial sale in 1982. It will be marketed as a 75% dry flowable formulation under the trade name GLEAN (12).



2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino-carbonyl]benzenesulfonamide

Figure 1. Chlorsulfuron chemical structure.

[1] At the time of writing, this common name has been approved by the Weed Science Society of America, but not by the Canadian Standards Association.

On the basis of its chemical structure (Figure 1) chlorsulfuron cannot be classified easily according to existing groups of herbicides. The molecule contains both a substituted urea and a substituted triazine core group.

Chlorsulfuron can be synthesized by reacting equivalent amounts of 2-chlorobenzenesulfonyl isocyanate with 2-amino-4-methoxy-6-methyl-1,3,5-triazine in acetonitrile. The final, desired product is an odourless, white, crystalline solid with a melting point of 174 - 178°C and a molecular weight of 357.8. Chlorsulfuron is moderately soluble in methylene chloride, ethyl acetate, and tetrahydrofuran; it is less soluble in acetone and acetonitrile, and it is of very low solubility in such hydrocarbon solvents as toluene and hexane (6,62). Its solubility in water is 125 ppm at 25°C. The vapour pressure of chlorsulfuron is 4.6×10^{-4} mm Hg at 25°C, which is low when compared to other herbicides (5).

Chlorsulfuron is a weak organic acid that has been observed to degrade by hydrolysis on storage in such protic solvents as methanol, ethanol, and acetone. The half-life is about one week in pH 4 buffer, one month at pH 7-9. The hydrolysis products are 2-chlorobenzenesulfonamide and 2-amino-4-methoxy-6-methyl-1,3,5-triazine. These products are readily separated by means of thin layer chromatography (31). DuPont reports that chlorsulfuron is subject to photolysis under sunlight in aqueous solutions (6). Under these conditions, half-lives of 2 to 4 weeks have been

observed. Chlorsulfuron is stable as a dry film on glass, but it is subject to photolysis on dry soil or on dry plant matter, with a half-life of 6 to 8 weeks.

Toxicological tests suggest that chlorsulfuron is of comparatively low mammalian toxicity. Its LD_{50} for fasted male rats is 5545 mg/kg. Chlorsulfuron is rapidly excreted by rats via the urine without undergoing extensive metabolism. Neither chlorsulfuron nor its hydrolysis or metabolism products have shown any potential accumulation through the food chain (6).

2.1.2 Herbicidal Behaviour

Field research in Western Canada in 1979 (71) has indicated that a postemergence application[2] of 50 to 100 g/ha of chlorsulfuron provides good control of such common broadleaved species as Tartary buckwheat, pale smartweed (*Polygonum lapathifolium* L.), lamb's quarters (*Chenopodium album* L.), hempnettle, stinkweed (*Thlaspi arvense* L.), wild buckwheat (*Polygonum convolvulus* L.), redroot pigweed (*Amaranthus retroflexus* L.), common chickweed [*Stellaria media* (L.) Vill.], henbit (*Lamium amplexicaule* L.), cleavers (*Galium aparine* L.), and Canada thistle [*Cirsium arvense* (L.) Scop.]. Most annual grasses, including wild oats (*Avena fatua* L.), are tolerant, except green foxtail [*Setaria viridis* (L.) Beauv.], whose growth can be suppressed by a

[2] Throughout this thesis, herbicide rates are recorded in grams or kilograms of active ingredient per hectare.

preemergence, incorporated treatment of 70 g/ha. The cereal crops are tolerant to chlorsulfuron. Flax (*Linum usitatissum*), field beans (*Phaseolus vulgaris*), lentils (*Lentilla lens*), and rapeseed are susceptible, especially when chlorsulfuron is applied in a preemergence, incorporated treatment (29).

The reports on 1980 herbicide field research in Western Canada indicate results similar to the ones obtained in 1979 (30). In general, in 1980 it was found that rates of chlorsulfuron as low as 10 to 40 g/ha gave acceptable results, especially if an adjuvant was added. At these lower rates, the control of such annual weed species as Tartary buckwheat, henbit, Russian thistle (*Salsola pestifer* A. Nels.) became marginal, especially at the later leaf stages. Stinkweed, rapeseed, redroot pigweed, and lamb's-quarters could be controlled with 5 g/ha of chlorsulfuron.

Levitt et al. (62) report that a postemergence application of chlorsulfuron at 1 g/ha in a 0.2% Tween 20 {oxysorbic (20ETO)[polyoxyethylated sorbitan monoleate (polysorbate 20ETO)]} solution effectively controlled such weeds as velvetleaf (*Abutilon theophrasti* Medic.), common morning glory (*Ipomoea purpurea* L.), and wild mustard [*Brassica kaber* (DC.) L.C. Wheeler]. Rates of 8 g/ha or higher were required to control cocklebur (*Xanthium strumarium* L.) and jimson weed (*Datura stramonium* L.). Severe wheat (*Triticum aestivum*) injury occurred at 120 g/ha. In preemergence applications of chlorsulfuron a rate

of 8 g/ha was needed to control wild mustard and common morning glory, while 16 g/ha was required to control velvet leaf. In order to control giant foxtail (*Setaria faberii* Herrm.) and cocklebur, 125 g/ha had to be applied. This high rate did not effectively control cheatgrass (*Bromus secalinus* L.) and crabgrass [*Digitaria sanguinalis* (L.) Scop.]. Neither wild oats nor wheat was severely affected by this high rate of chlorsulfuron. Other weeds that can be controlled by either a preemergence or a postemergence application of 16 g/ha are kochia [*Kochia scoparia* (L.) Schrader], false chamomile (*Matricaria inodora*), dog fennel (*Anthemis cotula* L.), and wild buckwheat.

On the basis of a worldwide review, Palm (72) concluded that winter and spring wheat and barley (*Hordeum vulgare*) have shown the greatest tolerance to preemergence treatments of chlorsulfuron. The yield of these crops commonly has not been reduced by rates as high as 100 g/ha. The tolerance to preemergence treatments is adequate for wheat but inadequate for barley. Winter wheat has shown no injury at 40 g/ha, applied either preemergence or early postemergence in the fall. Winter barley can tolerate postemergence treatments up to 30 g/ha.

Hageman and Behrens (42) evaluated the response of several spring wheat, barley, and oat cultivars to preemergence and postemergence applications of chlorsulfuron. In the greenhouse, preemergence applications of 1000 g/ha reduced the average height of the wheat, barley, and

oat (*Avena sativa*) cultivars by 69, 65, and 39%, respectively. There were significant differences among the wheat and barley cultivars. All treated plants had severe interveinal chlorosis. In the field, wheat, barley, and oat cultivars were tolerant to 250 g/ha of chlorsulfuron, applied preemergence, with no differential cultivar response. Chlorosis was observed at only one of the two locations.

Postemergence applications of 1000 g/ha in the greenhouse resulted in a 40 to 50% reduction in the height of wheat and barley cultivars, and a 20 to 30% height reduction for oat cultivars. Moderate interveinal chlorosis was observed in wheat and barley cultivars, but not in oat cultivars. Field evaluation of 250 g/ha chlorsulfuron postemergence application revealed the tolerance of all cultivars. No chlorosis or stunting was observed. The yield of durum wheat was reduced by a 250 g/ha postemergence application, but not by a 125 g/ha treatment.

Roberts and Bond (80) assessed chlorsulfuron for weed control in vegetable crops. Applying 5 g/ha of chlorsulfuron in either a preemergence spray application or a preplant-incorporation treatment, resulted in almost complete kill of all vegetable crops tested. A postemergence application of 40 g/ha to these crops at different growth stages also resulted in death or severe injury. The most tolerant species present were black nightshade (*Solanum nigrum* L.), annual bluegrass (*Poa annua* L.), perennial

ryegrass (*Lolium perenne* L.), bird's-eye (*Veronica persica* Poir), and earth smoke (*Fumaria officinalis* L.).

Richardson et al. (79) reported that chlorsulfuron at 40 g/ha severely damaged perennial rye grass seedlings. McAteer and Courtney (66) tested mature stands of a range of varieties within each of the main agricultural grass species for their tolerance to chlorsulfuron. Both the 20 and 40 g/ha treatment caused a marked reduction in the growth of perennial rye grass, Italian rye grass (*Lolium multiflorum* Lam.), and fescue (*Festuca elatior* L.) varieties. The growth of orchard grass (*Dactylis glomerata* L.) varieties was reduced by the 40 g/ha rate only.

When the herbicide antidote NA (1,8-naphthalic anhydride) is applied to corn seeds prior to planting, the developing corn seedlings will be protected from relatively high rates of EPTC (S-ethyl dipropylthiocarbamate) (51). Parker et al. (73) have shown that the tolerance of corn, sorghum (*Sorghum bicolor* Moench), and rice (*Oryza sativa*) to chlorsulfuron can be greatly increased by seed dressing with this herbicide antidote at 0.5% (w/w) of the seed weight. However, because of their high sensitivity to chlorsulfuron, these crop plants still suffered severe chlorsulfuron damage at 5 g/ha. NA does not offer any protection to chlorsulfuron in rapeseed, sugarbeets (*Beta vulgaris*), onions (*Allium cepa*), dwarf beans, and perennial rye grass. Wheat and barley are well protected by 0.5% NA. In barley, chlorsulfuron rates as high as 400 g/ha did not adversely

affect the shoot dry weight. This could offer possibilities for the control of such problem weeds as *Bromus* spp. DuPont suggests these species are of intermediate susceptibility to chlorsulfuron (6).

The influence of surfactants on herbicide efficacy has been investigated extensively (10,32,54). The herbicidal performance of chlorsulfuron could also be enhanced by the addition of non-ionic surfactants (18). The addition of 0.5% Agral 90 (90% alkyl phenol ethylene oxide condensate) to the chlorsulfuron (80% WP) spray solution, 5 g/ha, significantly reduced the fresh weight of rapeseed seedlings in a postemergence application. This reduction was less, although still significant, with the addition of either 0.5% Atplus 300F (80% polyoxyethylene sorbitan fatty acid ester), 0.5% Cittowet Plus (50% alkylaryl polyglycol ether), or 0.5% Renex-36 [Polyoxyethylene(6)tridecyl ether]. The concentration of Agral 90 in the spray solution could vary between 0.025% and 0.5% without significantly affecting the results.

Chow and Taylor (18) used five Ethylan (nonyl phenol ethylene oxide condensate) surfactants with HLB[3] numbers between 10.5 and 17.4, 0.25% of the spray solution, to determine the influence of surfactant character on the efficacy of chlorsulfuron. They found that the surfactant

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[3]The HLB number, ranging from 0 to 20, is a quantitative expression used to indicate the balance between the hydrophilic and lypophilic parts of a surfactant molecule. A high number indicates a more hydrophilic molecule (10).

with an HLB number of 17.4 increased the effect of a 5 g/ha application of chlorsulfuron (80% WP) on rapeseed seedlings the least. No significant differences were observed between the effect of surfactants with HLB numbers ranging between 10.5 and 16.0. Since the HLB numbers of Agral 90, Atplus 300F, Cittowet Plus, and Renex-36 are all within this range, Chow and Taylor concluded that the differences in their modification of chlorsulfuron efficacy cannot be accounted for by the differences in their HLB number.

The same authors also determined the amount of chlorsulfuron intercepted and retained by rapeseed seedlings by adding a fluorescent dye to the chlorsulfuron spray solutions. The rapeseed seedlings sprayed with a solution that contained Agral 90 had the largest amount of chlorsulfuron per unit dry weight. This observation may account for the greater increase in chlorsulfuron efficacy by Agral 90 than by the other three surfactants.

One of the most pronounced effects of chlorsulfuron on susceptible plants is growth inhibition. When young corn seedlings were placed in nutrient solutions containing 1 to 100 ppb of chlorsulfuron, net increases in the weights of shoots and roots were significantly reduced after 48 hr at all concentrations (77). Six hours after chlorsulfuron had been applied to the foliage of a young corn plant, the growth rate was only 50% of the control. The growth rate of resistant species remained unaffected.

Chlorsulfuron at concentrations of 100 ppm had no effect on O_2 evolution of isolated pea (*Pisum sativum*) and spinach (*Spinacia oleracea* L.) chloroplasts. The incorporation of $^{14}CO_2$ into the leaves of susceptible plants also was not affected by chlorsulfuron. The respiration rate of pea roots treated with 10 ppm of chlorsulfuron was identical to the control rates, for as long as 48 hr.

Chlorsulfuron at concentrations up to 10 ppm did not affect the indole acetic acid-induced elongation of etiolated pea stems, nor the cytokinin-induced cell expansion in cucumber (*Cucumis sativus*) cotyledons, nor did it affect the gibberellic acid-induced elongation of lettuce (*Lactuca sativa*) hypocotyls. Thus, the effect of chlorsulfuron on growth rate is not directly due to an effect on cell elongation or expansion processes in susceptible plants.

The cell division process appears to be extremely sensitive to chlorsulfuron. The mitotic index of broadbean (*Vicia faba*) root tips was reduced from 6.4 to 0.9 division figures per 100 cells after treatment with 1 ppm of chlorsulfuron. This was also demonstrated by the inhibition of ^{3}H-thymidine incorporation into treated corn roots. It is generally assumed that this test provides information on the amount of DNA synthesized in cells, which is a function of the amount of cell division occurring (11,16). Four hours after treatment with 10 ppb of chlorsulfuron, a 50% inhibition of ^{3}H-thymidine incorporation into corn root tips

could be observed. During this period, protein and RNA synthesis were not affected. Ray suggests that all these results indicate that the primary target site of chlorsulfuron is cell division (78).

In order to localize the site of action of herbicides, several techniques can be used (7,25,33,55). De Villiers et al. (22) studied the effect of chlorsulfuron on different plant biochemical processes, utilizing single, enzymatically isolated, leaf cells, and isolated chloroplasts. He concluded that the primary effect of chlorsulfuron in isolated leaf cells is on photosynthesis. Chlorsulfuron also inhibited such processes as respiration, RNA synthesis, lipid synthesis, and protein synthesis, but to a much smaller extent. This inhibition could be an indirect effect, since in isolated leaf cells these processes are dependent upon photosynthesis for energy supply. Since chlorsulfuron does not affect the light-induced ATPase activity of isolated chloroplasts, it is suggested that it inhibits photosynthesis by inhibiting PS II-mediated oxygen evolution (1,22). The specific site involved in this inhibitory action has not been determined. Ray's work (77) indicates that the oxygen evolution of chloroplasts incubated with 100 ppm of chlorsulfuron is not affected. Since the inhibition of photosynthesis, as reported by de Villiers, occurred at high concentrations of chlorsulfuron, 10^{-4} M, it is concluded that this is probably not the primary mode of action of chlorsulfuron. Most photosynthesis-inhibiting herbicides

exhibit their effect at much lower concentrations, i.e., 10^{-6} M (7).

Sweetser et al. (89) have investigated the basis for the selectivity of chlorsulfuron. The tolerant plants such as wheat, oat, and barley, rapidly metabolized chlorsulfuron to a polar, inactive product. This metabolite has been characterized as the *O*-glucoside of chlorsulfuron, in which the phenyl ring has undergone hydroxylation followed by conjugation with a carbohydrate moiety (31). No metabolism was observed in sensitive broadleaved plants. Black nightshade (*Solanum nigrum* L.) also metabolized chlorsulfuron, but to different products than wheat, oats, or barley.

2.2 Herbicide Persistence in Soil

It has been observed that chlorsulfuron is sufficiently persistent in the soil environment to affect the growth of rapeseed one year after application (12,30). DuPont submits that under growing-season conditions, the half-life of chlorsulfuron in the soil is 4 to 6 weeks (6). Because no detailed study on the degradation of chlorsulfuron has been reported yet, the intent of this section is to review some of the factors that influence herbicide persistence in soil. Some extensive reviews (41,45,48,64) have been published recently on this topic. This synopsis follows closely the discussion by Hurle and Walker (53).

The persistence of a herbicide in the soil is dependent upon its rate of loss from the soil environment. This loss can occur by means of such processes as volatilization, photodecomposition, leaching, chemical breakdown, or microbial degradation (64). Since the products of the degradation process might be phytotoxic themselves, an understanding of the degradation pathway(s) is crucial.

The rate of loss of a herbicide from the soil can be affected by the initial concentration of the herbicide. Both for herbicides and for insecticides (28) it has been found that at unusually high rates of application, the persistence can increase disproportionately. At recommended rates of application this effect has not been observed, and the rate of loss is independent of the initial concentration. In the case of some herbicides, an initial lag phase has been observed. During this phase no herbicide loss is recorded. The duration of the lag phase can depend on the initial concentration of the herbicide in the soil.

The influence of soil type on the rate of loss of a herbicide is not well understood. Reports in the literature have presented conflicting results. It is suggested (43) that an increase in organic matter might increase the rates of degradation in mineral soils up to a limiting value, above which the rate of loss would be reduced. The effect of herbicide adsorption on the rate of loss is also poorly understood. Herbicides can be adsorbed by both the soil particles and the organic matter, reducing the concentration

in the soil solution. However, this adsorption is not necessarily a protection. The microbial activity is greater at the adsorption surfaces than in the soil solution (15). Besides, the adsorption sites may catalyze non-biological reactions. The adsorption of chlorsulfuron to clay is low. Organic matter appears to have greater affinity for this herbicide (6).

The effect of soil pH on the rate of loss can be either direct or indirect. A direct effect occurs when the stability of the herbicide is pH-dependent; an indirect effect occurs when the pH affects the adsorption process or the composition of the soil microflora. Although very little is known about the principles involved in these processes, it has been suggested (19) that the maximum rate of loss of a herbicide occurs at those pH levels that are favourable for the growth of the specific microorganisms involved in the microbial degradation.

Both temperature and moisture are important factors in determining the rate of loss of a herbicide from the soil. In general, an increase in soil temperature results in an increase in the rate of loss, due to increases in rates of non-biological reactions and biological processes. There is also evidence that the rate of loss increases with increasing soil moisture content, up to field capacity. In the cases of picloram (4-amino-3,5,6-trichloropicolinic acid) and atrazine (2-chloro-4-ethylamino-6-isopropylamine-S-triazine) it has

been demonstrated that the rate of loss under cool, dry conditions is significantly lower than under hot, wet conditions (44,47).

The rate of loss of a herbicide from the soil is dependent upon its distribution within the soil. At the surface, losses due to volatilization and photodegradation are expected to be high. Since microbial activity in the topsoil is greater than in the subsoil, the rate of loss in the former is usually greater than in the latter. Field experiments have verified the validity of these principles.

Long-term experiments with repeated applications of such herbicides as linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], triallate [S-(2,3,3-trichloroallyl)diisopropylthiocarbamate], simazine [2-chloro-4,6-bis(ethylamine)-S-triazine], and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] indicate that there is no accumulation of residues in the soil. This would suggest that the use of herbicides does not adversely affect the rate of loss of subsequent applications. Repeated applications of such herbicides as 2,4-D, MCPA (2-methyl-4-chlorophenoxyacetic acid), DNOC (4,6-dinitro-*o*-cresol), and chlorpropham (isopropyl-*m*-chlorocarbanilate) have shortened the initial lag period before degradation would begin, presumably due to an adaptation of the microbial population.

The effect of a crop on the rate of herbicide loss depends indirectly on soil and environmental factors. No general principles have been established.

The persistence of a herbicide in the soil may be affected by its formulation. In the case of insecticides the relative order of persistence was granules > emulsions > miscible liquids > wettable powders. Although this order of persistence has not been fully verified for herbicides, it is generally accepted that granules are more persistent than any other formulation, especially with such volatile herbicides as triallate, propham (isopropyl carbanilate), and EPTC.

The rate of loss of a herbicide from the soil can also be affected by the presence of another pesticide. Both increases and decreases in the rate of loss of several herbicides have been observed. Usually it is suggested that the impact of the second pesticide on the microbiological degradation processes is responsible for the change in rate.

In spite of the many complex factors that govern herbicide persistence in the soil, and in spite of many unsuccesful attempts to describe the kinetics of the herbicide degradation processes adequately, generalized predictions of herbicide losses have been surprisingly good. Using a computer model, Walker (94) was able to predict accurately the simazine residues over a 4-year period after annual preemergence applications of 1.68 kg/ha to maize and 3.36 kg/ha biannual applications to vegetation-free plots. In a similar experiment this computer model was able to predict satisfactorily the persistence of asulam (methyl sulfanilylcarbamate) in five of the six trials (88).

2.3 Tartary Buckwheat

Tartary buckwheat is a glabrous, annual plant, 50 - 100 cm high, that spreads by seed. Its erect, green stem bears petioled, light-green, alternate leaves that are triangular-heartshaped, with widespread lobes at the base. The lower leaves are longer-stalked than the upper ones. The base of the leaf stalk is covered with an oblique, entire ocrea. The greenish-white, apetalous, perfect flowers are borne in small, clustered racemes at the ends of stems and in the leaf axils. Each flower has eight stamens, three styles, and an equally five-parted calyx. The sharply three-sided seed protrudes from the sepals; it is brownish or gray, rough and dull, and about 5 mm long. Tartary buckwheat usually flowers in the early summer and its seed matures over a rather extended period (13,34,39,63,69,86). It differs from common cultivated buckwheat (*Fagopyrum sagittatum* Gilib.) in that the latter has larger, white or reddish flowers and smoother, shining, more sharply triangular seeds.

Tartary buckwheat is believed to have originated in central Asia from the species *Fagopyrum cymosum*, which is found in the Himalaya (17). Its distribution in Canada is not well understood (34). It occurs in the eastern provinces, but only in north-central Alberta is it regarded as a weed. In New Brunswick, Tartary buckwheat has been grown extensively as a crop plant. In the hilly regions of India, Tartary buckwheat has promised to be an excellent

alternative food crop, due to its great adaptability and the high caloric value of its flour (17).

In Alberta, Tartary buckwheat is regarded as a nuisance weed (3). According to a recent weed survey (23), it is predominantly found in a 150-km wide strip ,stretching latitudinally between Edmonton and the Alberta-Saskatchewan border. The infestation in this region is classified as light, i.e., 1 to 8 plant/m^2. Tartary buckwheat is especially troublesome in wheat, since its seeds are similar in size and weight to the wheat kernels, making separation difficult.

At the time of maturity, the seeds of Tartary buckwheat are dormant. This dormancy cannot be broken by scarifying the fresh seeds with such chemicals as concentrated sulfuric acid, 95% ethanol, or gibberellic acid (10 - 10,000 ppm) (92). A partial removal of both the pericarp and the seed coat did not overcome dormancy either; only a complete removal of these structures resulted in an increase in germination of fresh seed. Dormancy can be broken also by storage of the seeds at room temperature for relatively short periods, i.e., 28 days (2). This afterripening process can be accelerated by a higher temperature, i.e., 40°C (92).

Germination is also influenced by temperature. Field studies have indicated that the period required to attain maximum germination increases with decreasing soil temperatures (93). In the field, seedlings of Tartary buckwheat can emerge from a depth of 10 cm (2). Tartary

buckwheat usually flowers 5 to 6 weeks after the onset of germination. The first seeds are mature about 4 to 5 weeks later. Due to the indeterminate flowering habit of Tartary buckwheat, flowers, immature, and mature seeds may all be present on the same plant at any given time after the onset of flowering. The number of seeds per plant can vary between 400 and 1100.

The Alberta recommendations for chemical weed control (4) list several herbicides that can be used to control Tartary buckwheat in cereal crops and flax. This list includes such herbicides as MCPA, dicamba (3,6-dichloro-*o*-anisic acid), cyanazine [2-chloro-4-(1-cyano-1-methylethylamino)-6-ethylamino-*s*-triazine], bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), linuron, metribuzin [4-amino-6-*tert*-butyl-3-(methylthio)-as-triazin-5(4*H*)-one], propanil (3',4'-dichloropropionanilide), picloram + 2,4-D [(2,4-dichlorophenoxy)acetic acid], dichlorprop [2-(2,4-dichlorophenoxy)propanoic acid] + 2,4-D, and some combinations of these. In the light of these recommendations it can be stated that Tartary buckwheat does not constitute a serious threat to agricultural production in Alberta.

2.4 Cell Division

2.4.1 Cell Cycle

The growth and development of higher plants is a continuous process that comes to expression by means of cell division, cell expansion, and cell differentiation. The study of the cell division phenomenon has developed from a mere description of the mitotic processes to an understanding of the complete cycle a cell passes through (14). This cell cycle has been defined as the period of time from the formation of a cell by the division of its 'mother cell', until that cell itself divides to form two new cells (84). Largely due to the developments in cell biology, we can now distinguish between four phases in this cell cycle (60,95). These phases are: G1, the pre-DNA synthesis phase; S, the DNA synthesis phase; G2, the pre-mitotic phase; and M, the phase during which mitosis and cytokinesis occur. The G1, S, and G2 phases together constitute what traditionally has been known as interphase.

Dividing cells pass through these phases in sequence. RNA and protein synthesis occur in almost all phases, but at different rates (95). The rate of RNA and protein synthesis is reduced during the mitotic phase. DNA synthesis occurs predominantly in the S phase; a phase during which RNA and protein synthesis also occur at a high rate. At the end of the S phase the total amount of DNA has doubled (96).

Except for the G1 phase, the duration of each phase is more or less fixed. Cells that do not divide anymore remain in the G1 phase. In dividing cells the triggering event for all the subsequent phases of cell division appears to occur during this G1 period. Once the S phase begins, the subsequent phases of the cycle usually follow without delay (95).

The study of the action of chemicals on dividing cells has contributed to the understanding of the cell cycle (58). Chemicals can act on the cell cycle in one of three ways (20,83). (i) They may arrest the cell cycle at some stage of the interphase, affecting processes in either the G1, S, or G2 phase. Examples of these pre-prophase inhibitors are actinomycin D, chloramphenicol, cycloheximide, 3'-deoxy-adenosine, and hydroquinone (58). Their action is concentration-dependent, and at high concentrations some of these inhibitors also act in other possible ways. (ii) Some chemicals interfere with the synthesis or the orientation of the mitotic spindle. These are the so-called metaphase poisons (58) or mitoclasic agents (83), of which colchicine is a prime example. (iii) Inhibitors of cytokinesis inhibit cell plate and cell wall formation. Caffeine has been observed to produce these effects, resulting in binucleate cells (38).

The cell division process can also be affected by a reduction in energy supply (58). Chemical energy is needed during both the mitotic phase and the interphase.

Consequently, oxidative phosphorylation inhibitors and uncouplers, i.e., dinitrophenols and oligomycin, respectively, will suppress cell division (60).

2.4.2 Effect of Herbicides

The mode of action of several herbicides can be attributed to their deleterious effect on cell division (67). Again we can distinguish between those herbicides that interfere with the formation and function of the mitotic spindle, or the formation of the cell plate and cell wall, and those that exert their influence predominantly during the interphase period of the cell cycle. These last ones can either interfere with the synthesis or active transport of precursors into the nucleus that are required for DNA, RNA, and protein synthesis, or they can modify the chemical and physical properties of these macromolecules and their complexes.

The morphological, anatomical, and mitotic aberrations produced by the herbicides trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), oryzalin (3,5-dinitro-*N*⁴,*N*⁴-dipropyl-sulfanilamide), pronamide [*N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide], propham , and chlorpropham are similar to those observed with colchicine (9,91), i.e., inhibition of root elongation, swelling in the elongation zone, delay in centromere division, inactivated spindle apparatus, and arrest of cell division at metaphase. Despite this similarity, the mode of action of these

chemicals appears to be different. Bartels and Hilton (9) concluded that the dinitroaniline herbicides trifluralin and oryzalin and the chlorobenzamide herbicide pronamide interfere with the synthesis and function of the microtubule subunits. The phenylcarbamates propham and chlorpropham act on the microtubule organizing centre. Colchicine seems to inhibit the polymerization of the subunits into the microtubules.

The herbicide ioxynil (4-hydroxy-3,5-diiodo-benzonitrile) has been reported to be an uncoupler of oxidative phosphorylation (57,84). The resulting decline in available ATP can have a suppressing effect on cell division. Rost et al. (84) found that a low concentration of ioxynil, 1 μM, acted as a pre-prophase inhibitor of cell division in pea roots. This may indicate that the primary biochemical action of ioxynil is not necessarily related to the effect on oxidative phosphorylation. This example illustrates the necessity of considering dose responses to herbicide treatments, also in mode of action studies. D'Amato et al. advocated this already in 1960 (20).

The syntheses of DNA, RNA, and protein are important processes in the cell cycle. Usually the effect of a herbicide on these processes is determined by the incorporation of radiolabelled precursors into DNA, RNA, and protein by excised plant tissue, e.g., root tips (65,67,68). Ashton et al. (7) have developed a method to study these processes using single, enzymatically isolated, leaf cells.

The dependency of DNA, RNA, and protein synthesis on chemical energy has complicated the study of the inhibitory action of herbicides on these processes. Gruenhagen et al. (40) have established correlations between the inhibition of RNA and protein synthesis and reduced tissue ATP concentrations. They found that the strongest inhibitors of RNA and protein synthesis were also the herbicides that reduced the tissue ATP content significantly. Similarly it was found that the inhibition of RNA and lipid synthesis in isolated cotton and soybean leaf cells by the herbicide SAN 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone] is an indirect result of an inhibition of photosynthesis (76).

A direct effect upon DNA and RNA synthesis has been reported for trifluralin (74), atrazine (75), and the experimental herbicide WL 29226 [4-(2,6-dichlorobenzoyl-oxymethyl)-4-ethyl-2,2-dimethyl-1,3-dioxolan] (16). In these instances the herbicides interacted with the chromatin. Chromatin consists mainly of approximately equal amounts of DNA and histone proteins (60). The histones are small, basic proteins rich in lysine and arginine. They appear to be of fundamental importance in the structural organization of chromatin. Although their function as specific gene repressors remains in question, they do play a role in regulating transcription (95).

It has been shown that atrazine applied to 4-day old etiolated soybean seedlings 6 hr before isolation of

chromatin, enhanced the chromatin-directed RNA synthesis (75). This is probably due to increased template availability. Trifluralin applied to etiolated corn seedlings 6 to 12 hr before the isolation of chromatin from the roots reduced the chromatin-directed RNA synthesis (74). This is attributed to a possible binding of trifluralin to the chromatin, with a subsequent reduction in template availability for transcription.

Burrows (16) reported that WL 29226 reduced the level of RNA synthesis in 60-hr old wheat seedlings. Since this reduction could not be accounted for by the reduction in uptake of precursor molecules alone, the herbicide is considered to have a direct effect on nucleic acid biosynthesis. Monitoring the DNA polymerase activity on chromatin isolated from herbicide-treated maize and barnyard grass, it was found that the activity was stimulated in barnyard grass and reduced in maize chromatin. The failure of WL 29226 to induce an inhibition *in vitro* on isolated chromatin does not suggest a direct interaction between the herbicide and chromatin. It appears that this herbicide alters the integrity of the chromatin.

2.4.3 Effect of Hydroxyurea

Hydroxyurea is an effective inhibitor of DNA synthesis, but it has little effect on the synthesis of RNA and of protein (59,81). The effect on DNA synthesis is easily reversible by transferring the treated tissue to a growth

medium free of hydroxyurea. Young and Hodas (97) suggest that hydroxyurea interferes with the reduction of ribonucleotides to deoxyribonucleotides due to the inhibition of the enzyme ribonucleotide reductase (37,46,90). Rosenkranz et al. (82) observed that in the bacteria *Escherichia coli* the hydroxyurea-induced inhibition of DNA synthesis is immediate. This indicates that either hydroxyurea interferes with a rate-limiting enzymatic reaction concerned with DNA synthesis or it reacts rapidly with the DNA, preventing it from serving further as a template for DNA synthesis.

Cytological investigations have shown that cells that were in the S phase while being exposed to hydroxyurea were lethally damaged. G1 phase cells survived but were prevented from beginning DNA synthesis. G2 cells also survived and progressed in their development to the end of the G1 phase (87). Even though hydroxyurea cannot be classified as a metaphase poison as such, chromosomal aberrations have been observed in broadbean root tips after 6 hr in 10^{-3} M hydroxyurea. These aberrations are similar to the ones obtained after treatment with inhibitors of deoxyribonucleotide synthesis, suggesting a similarity in action (59).

Barlow (8) observed that the time needed to reduce the mitotic index of hydroxyurea-treated corn root tips to zero was approximately equal to the duration of the G2 period. Although he did not report the actual length of this period,

it is known that, depending upon their location, the cells in the corn root tips divide every 12 to 27 hr. Experiments with bacteria have shown that the rate of ^{3}H-thymidine incorporation into hydroxyurea-treated cells decreased significantly 4 to 5 min after the onset of the treatment (82). In the case of broadbean root tips a 3-hr treatment with 10^{-3} M hydroxyurea resulted in a 76% inhibition of ^{32}P-incorporation into DNA (59). These results indicate that hydroxyurea acts rapidly.

Because of its specific action on DNA synthesis, and the resulting inhibition of cell division, hydroxyurea has been used to study growth in the absence of cell division. Barlow (8) used 10^{-2} M hydroxyurea to determine whether cells in different regions of the root meristem of corn have intrinsically different rates of growth. Hoppe (52) studied the interaction between hydroxyurea and diclofop methyl {methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate} on the radicle growth of corn seedlings, in order to elucidate further the mode of action of the latter compound.

3. MATERIALS AND METHODS

3.1 General Procedures

3.1.1 Field Experiments

During the spring and summer of 1980 and 1981 nine field experiments were conducted at four different sites in north-central Alberta. Table I presents detailed information on each site. In all experiments the experimental design was a randomized block. All treatments, including the weedy control treatment, were replicated four times, except at the St. Albert site where only three replicates were used. Barley, wheat, wild oats, and Tartary buckwheat were seeded with a press drill at 15-cm row spacing. Rapeseed was seeded at 22.5-cm row spacing using an experimental-plot seeder.

Table I. **Information on Field Experiment Sites.**

Site	Soil texture	pH†	O.M. %	Sand %	Silt %	Clay %	Plot size m
Ellerslie	loam	5.9	14.3	38	47	15	1.8 x 5.4
Neerlandia	sandy loam		33.3	48	44	8	3.0 x 3.6
Villeneuve	clay loam		10.3	31	41	28	3.0 x 3.6
St. Albert	si.cl.loam	6.0	9.6	13	58	29	3.9 x 6.0

† Determined with distilled water method.

Herbicide applications were made with a bicycle-type plot sprayer equipped with TeeJet 8001 nozzle tips. The spray volume was 100 L/ha and the spray pressure was 276

kPa. Spray nozzle height was adjusted to 50 cm above the target. In the case of postemergence wild oat herbicides the spray angle was 45° forward; in all other instances the spray was directed straight down.

Throughout the growing season all experiments were rated visually for crop injury and weed control. At the time of harvest, the grain yield and the dry weight[4] of the weed(s) per m^2 were determined. In the case of rapeseed, the dry weight instead of the yield was determined. Statistical analysis of the field data consisted of an analysis of variance, and, if the F-value was significant at the 5% level, a Duncan's New Multiple Range Test (26).

3.1.2 Controlled Environment Experiments

The controlled environments used in this study were a greenhouse, a growth chamber, and an incubator. In the greenhouse, plants were grown in individual pots filled with a sterilized 2:1:1 soil mix (clay-loam:peat:sand). The plants were watered twice daily. The temperature in the greenhouse was maintained at 20±2°C. During the summer, higher readings have been observed. Relative humidity was 40 to 60%. The greenhouse daylength was extended to 16 hr with a combination of metal halide and high pressure sodium lights. The average photon flux density was 300 $\mu E.m^{-2}.s^{-1}$ (measured with a Li-Cor quantum meter, Model Li-185; Lambda

[4]Throughout this thesis, dry weight refers to the weight of plant material after it has been dried at 70 to 80°C for 24 to 48 hours.

Inst. Corp., Lincoln, Nebraska).

In the growth chamber, Tartary buckwheat plants were grown in styrofoam cups (200 ml) filled with horticultural vermiculite. To obtain a uniform stand, pre-germinated Tartary buckwheat seeds with a 1 to 1.5-cm long radicle were planted 1 cm below the vermiculite surface. The cups, which had five small holes in the bottom, were subirrigated with half-strength No. 1 Hoagland solution, modified to contain 1 ppm iron. The reservoir was a wooden tray (77 x 57 x 6 cm inside dimensions) lined with a clear plastic bag and a hardboard lid with 48 holes, sized to accommodate the styrofoam cups. Because no light could reach the nutrient solution, the growth of algae in the reservoir was prevented.

In order to prevent cross-contamination through the nutrient solution following a herbicide application, the styrofoam cups with the plants were placed in individual nutrient reservoirs, fitted in the tray under the lid, before the plants were treated. The cups remained in these small reservoirs for the duration of the experiment. The nutrient solution levels in the reservoirs were checked daily.

The temperature in the growth chamber was 25±1°C during the 16-hr day and 21±1°C during the night. A combination of fluorescent and incandescent lights supplied a photon flux density of 220 $\mu E.m^{-2}.s^{-1}$ The relative humidity ranged between 40 and 50%.

The temperature in the incubator (Percival, model E-30B) was 25±1°C. The 24-hr day was obtained with a combination of incandescent and fluorescent lights, supplying a 270 $\mu E.m^{-2}.s^{-1}$ photon flux density. The relative humidity was maintained between 60 and 70%.

All herbicide treatments requiring spray application were made with a specially designed pot sprayer. Resembling field spraying conditions, the travelling nozzle (TeeJet E8001) was calibrated to deliver 110 L/ha at 276 kPa pressure in a single pass over the plants. The nozzle height was adjusted to 50 cm above target.

The experimental design of all experiments performed in the controlled environments was a randomized block. Unless otherwise noted, six replicates were used in all instances.

3.2 Control of Broadleaved Weeds

In order to evaluate the control of Tartary buckwheat with chlorsulfuron[5], field experiments were conducted at the Ellerslie site in 1980 and 1981. In both years, 24 rows of Tartary buckwheat were seeded (75 kg/ha) across the front part of the plots, and several rates of chlorsulfuron were applied at different growth stages. The 1980 experiment included several preemergence treatments. In the 1981 experiments the treatments were applied with or without the surfactant Cittowet Plus (0.5% of spray volume). In 1980,

[5] Unless otherwise noted, the 75% DF formulation of chlorsulfuron has been used throughout this study.

the crop was barley (cv. Galt), seeded at 65 kg/ha; in 1981 the crop was wheat (cv. Neepawa), seeded at 58 kg/ha.

The control of hempnettle was evaluated at two different sites in 1980. The Neerlandia site was a farmer's field with a dense, natural infestation of hempnettle. The crop was barley (cv. Fairfield), seeded at 70 kg/ha. The treatments included three rates of chlorsulfuron and several other herbicides and herbicide combinations. The St. Albert site was a fallow field with a dense, natural infestation of hempnettle. The treatments included several rates of chlorsulfuron, MCPA, and a combination of chlorsulfuron and MCPA.

The Villeneuve site was the location of the cleaver control experiment, conducted in 1980. This site was a farmer's field with a light, natural infestation of cleavers. The crop was barley (cv. Bonanza), seeded at 65 kg/ha. The treatments were identical to the ones of the hempnettle control experiment at the Neerlandia site.

3.3 Influence on the Growth of Tartary Buckwheat

In the greenhouse, individual Tartary buckwheat plants were grown in 15-cm diameter pots. At the 3-leaf stage, all plants were sprayed with 0 or 20 g/ha of chlorsulfuron (80% WP) and 0.5% (v/v) adjuvant Atplus 411F (83% paraffin oil and 17% non-ionic ethoxylated ester surfactant; Atkemix Inc., Brantford). After the treatment, at predetermined time

intervals over a 21-day period, the height of one Tartary buckwheat plant per treatment was measured and the dry weight of the above-ground portion of the shoot was determined.

In the growth chamber, individual Tartary buckwheat plants were treated with eight different amounts of chlorsulfuron, ranging from 0 to 40 ug/plant, when they had reached the 1, 2, 3, or 4-leaf stage. Using an adjustable pipettor, a 10-ul droplet of the appropriate herbicide solution was placed on the adaxial surface of the youngest, fully expanded leaf in each instance. In order to ensure that only non-hydrolyzed chlorsulfuron was applied to the plants, technical chlorsulfuron (99% pure) was dissolved in tetrahydrofuran. Immediately prior to application, appropriate amounts of the stock solution were mixed with tetrahydrofuran and water containing the adjuvant Canplus 411F (83% paraffin oil and 17% non-ionic ethoxylated ester surfactant; Atkemix Inc., Brantford). The final treatment solution consisted of 20% tetrahydrofuran, 79.5% water, 0.5% Canplus 411F (v/v/v) and the required chlorsulfuron concentration.

During the 10-day period following application, detailed observations were made regarding the number of leaves, development of chlorosis, development of axillary leaves, stem shriveling, and the development of reproductive structures. Fourteen days after treatment the vermiculite was washed off the roots and the dry weight of the roots and

of the shoots was determined. These dry weights were analyzed as a split-plot; the leaf stages at the time of application were treated as the main plots, the chlorsulfuron dosages as the subplots. Within each main plot, each subplot was replicated six times according to a randomized block experimental design.

The effect of the adjuvant Cittowet Plus on the efficacy of chlorsulfuron was assessed in a separate experiment in the growth chamber. Individual Tartary buckwheat plants were sprayed with several rates of chlorsulfuron, with or without Cittowet Plus (0.5% of spray volume). The shoots of the plants were harvested 18 days after treatment and their respective dry weights were determined. These weights were analyzed factorially.

The amount of chlorsulfuron intercepted by Tartary buckwheat plants, 2 and 4-leaf stage, was determined by adding a red dye (2 g/L; Aldrich, 21074-9, Acid Red 4) to the spray solution. The rate of chlorsulfuron application was 20 g/ha. Immediately after spraying, the shoots of the plants, ten per leaf stage, were cut into several parts, placed in a test tube with 25 ml water, and shaken for 30 sec on a vortex mixer. The plant parts were removed and the solutions were adjusted to equal volumes. Spectrophotometric analysis of aliquots of the solutions at 508 nm was done with a double beam spectrophotometer (Beckman model 25) using the appropriate reference solution. A standard curve was established for absorbance vs ug of chlorsulfuron in the

solution. The dry weight of the shoot of each plant was determined and the amount of chlorsulfuron retained per plant or per mg dry weight was calculated.

3.4 Interaction with Wild Oat Herbicides

3.4.1 Field Experiments

The interactions between chlorsulfuron and postemergence wild oat herbicides were evaluated in experiments at the Ellerslie site in both 1980 and 1981. In both years, 24 rows of wild oats were seeded (42 kg/ha) across the front part of the plots and two rows of Tartary buckwheat (75 kg/ha) were seeded across the back. In 1980, the crops were barley (cv. Galt), seeded at 65 kg/ha, and wheat (cv. Neepawa), seeded at 58 kg/ha. The crop in 1981 was wheat (cv. Neepawa), seeded at 58 kg/ha.

In both 1980 and 1981, treatment applications were made at the leaf stage recommended for the wild oat herbicide. In 1980 two rates of chlorsulfuron were used, in 1981 only one.

3.4.2 Greenhouse Experiment

The effect of three rates of chlorsulfuron on the efficacy of postemergence wild oat herbicides was studied in a greenhouse experiment. Wild oats were seeded in 15-cm diameter pots filled with soil. One week after emergence the plants were thinned to seven uniform wild oat plants per

pot. The herbicide spray applications were made at the leaf stage recommended for the particular wild oat herbicide. The wild oat plants were harvested 40 days after treatment and their dry weights were determined.

This experiment was designed as a split-plot. The five wild oat herbicides were the main plots, the four rates of chlorsulfuron the subplots. Within each main plot, each subplot was replicated four times according to a randomized block experimental design. An analysis of variance of the dry weight data was done accordingly.

3.5 Tolerance of Cereals

In each field experiment that involved barley or wheat as the crop, a record was kept of the occurrence of crop injury after foliar treatment with chlorsulfuron. In order to evaluate the effect of high rates of chlorsulfuron on wheat, barley, and oats, a greenhouse experiment was conducted. Wheat (cv. Neepawa), barley (cv. Galt), and oats (cv. Cascade) were seeded in 15-cm diameter pots filled with soil. One week after emergence the plants were thinned to nine uniform plants per pot. The herbicide spray applications were made when the plants had three leaves. All chlorsulfuron treatments were applied in a 0.5% Atplus 411F solution. At 2, 9, 16, and 24 days after treatment one plant of each pot was harvested. The leaf stage and the total height of this plant were recorded and its dry weight was

determined. The remaining plants were observed until 70 days after treatment.

For each species the experimental design was a split-plot. The harvest times were the main plots and the rates of chlorsulfuron the subplots. Within each main plot, each subplot was replicated five times according to a randomized block experimental design. An analysis of variance was done accordingly.

3.6 Carryover in Soil

3.6.1 Effect on Rapeseed Crop

The 1980 experiment designed to evaluate the control of Tartary buckwheat in barley with chlorsulfuron at the Ellerslie site, became the 1981 experiment designed to assess the effect of chlorsulfuron carryover on rapeseed. In 1980, all the above-ground barley and Tartary buckwheat residue was removed and the site was rototilled twice to a depth of 10 cm. Soil samples for chlorsulfuron residue analysis were taken at this time. In the spring of 1981, the seedbed was prepared with a double disc and drag-tooth harrows. In order to minimize mixing of the soil among the plots, all tillage operations were done parallel to the longest side of the plots. Rapeseed (cv. Candle) was seeded at a rate of 6 kg/ha.

In 1979, the St. Albert site was the location of an experiment that evaluated the control of hempnettle in barley with chlorsulfuron. After the harvest in 1979, all the above-ground crop and weed residue was removed and the site was cultivated. In the spring of 1980, the plots that had received chlorsulfuron in 1979 were rototilled with a garden rototiller to a depth of 7 cm. Rapeseed (cv. Candle) was seeded at a rate of 7 kg/ha. The plots that had not received chlorsulfuron in 1979 became the location of the 1980 hempnettle control experiment at the St. Albert site.

In the fall of 1980, the above-ground residue of the chlorsulfuron carryover experiment and of the hempnettle control experiment was removed. The sites were subsequently cultivated twice. In the spring of 1981, both sites were double-disced twice and rapeseed (cv. Candle) was seeded at a rate of 7 kg/ha.

3.6.2 Corn Root Bioassay

Soil samples were taken from the plots of the 1980 Tartary buckwheat control experiment at the Ellerslie site, on November 3, 1980. From each plot, three random samples were obtained and bulked. For each sample, the topsoil to a depth of 10 cm from an area of approximately 300 cm^2 was collected. Untreated soil was obtained by a similar procedure from the area around the experiment site that had not received any chlorsulfuron. The control soil and the samples were air-dried in the greenhouse and subsequently

stored at 2°C. Prior to analysis all the soil was ground in a roller mill to pass through a 2-mm screen.

In March 1981, the concentration of chlorsulfuron in each sample was determined by a corn root bioassay. In this assay, a styrofoam cup was filled with 100 g of sample soil and one pre-germinated corn seed (cv. Pioneer L3369, F1 generation; Pioneer Hi-Bred International, Inc., Des Moines, Iowa) with a radicle length of approximately 1 cm was placed in each cup, 1 cm below the soil surface. After adding 25 ml water (pH 7.5), each cup was covered with aluminum foil and placed in the growth chamber. When the corn shoot had emerged, a hole was made in the aluminum foil allowing the shoot to protrude. Because the total weight of each cup was kept at approximately 130 g by the daily addition of water (pH 7.5), the initial soil moisture level was maintained throughout the duration of the assay. Nine days after the cups had been placed in the growth chamber, the soil was carefully washed off the roots and the length of the longest root was determined.

The standard curve was obtained by a similar procedure. In this instance, 100 g of control soil was thoroughly mixed with 25 ml of the appropriate chlorsulfuron solution to obtain the desired concentration. These concentrations were calculated on the basis of air-dried soil. Six replicates were used, both for the standard curve determination and for the analysis of the samples. In order to analyze the data, all corn root lengths were expressed in terms of percentages

of the corn root length obtained in soil without chlorsulfuron. A standard curve with its 95% confidence limits was drawn, relating root growth in terms of percent of control root length to the chlorsulfuron concentration in the soil. For each sample, the mean and the standard error were calculated. Using the standard curve, the corresponding concentration of chlorsulfuron, and its range on the basis of two times the standard error and the 95% confidence bands, were determined. Samples that had a chlorsulfuron concentration beyond the sensitive range of the bioassay, were diluted with appropriate amounts of control soil. The chlorsulfuron concentration of this mixture was multiplied by the dilution factor in order to obtain the concentration in the original sample. The mean chlorsulfuron concentrations were analyzed factorially.

3.7 Effect on Corn Root Growth

Corn seeds (cv. Pioneer L3396), surface-sterilized by soaking in 70% ethanol for 3 min and rinsed with sterile water, were germinated between sterile, moist paper towels for 72 hr at 25°C in the dark. In order to study the interaction between chlorsulfuron and hydroxyurea, five seeds, with 10 to 15-mm long radicles, were placed in a disposable growth pouch (Canlab B1220) that contained 35 ml of the appropriate herbicide solution. Four concentrations of chlorsulfuron, 0 to 10 ppb, with or without 10^{-2} M of

hydroxyurea, were assessed. Close contact between the growing corn roots and the moist paper towel in the pouch was maintained by keeping the pouch between two pieces of cardboard, of similar size as the pouch, and by placing them at an angle, 20° from the vertical, in the incubator. At the start of the experiment and at six 12-hr intervals, the location of the main root tip was marked on the transparent pouch and the distance to the previous mark was recorded. For each 12-hr interval the data were analyzed factorially.

3.8 Absorption and Translocation in Tartary Buckwheat

3.8.1 ^{14}C-Chlorsulfuron Experiment

The radioactive chlorsulfuron used in this experiment had a ^{14}C-label uniformly distributed in the phenyl ring of the molecule (Figure 1). Its specific activity was 222.37 Bq/ug.

The experiment was conducted in the growth chamber. At treatment time the Tartary buckwheat plants were in the 2-leaf stage and had been selected visually for uniformity.

Application procedure

Prior to applying the radioactive chlorsulfuron to the plants, the amount required for each of the four replicates was transferred from the stock vial to 1-ml cryogenic ampoules (Fisher 6-400A) using anhydrous ethyl acetate as solvent. After evaporating the ethyl acetate, the ampoules

were flame-sealed and stored at 2°C.

At the time of application the ampoules were opened and both tetrahydrofuran and an adjuvant solution were added to produce a final ^{14}C-chlorsulfuron solution consisting of 20% tetrahydrofuran, 79.5% water, and 0.5% Canplus 411F (v/v/v). A 10-µl droplet of this solution, containing approximately 1578 Bq of activity, was applied to the adaxial surface of the second true leaf of each plant. The exact amount of activity applied to each plant in one replicate was determined by putting a similar droplet in a liquid scintillation vial and counting it as outlined below. Another 10-µl droplet was saved in order to determine the chemical purity according to the method outlined in section 3.9.

Harvest procedure

At different times, 1 to 240 hr after treatment, one plant of each replicate was harvested. The treated area was washed twice. In each instance one end of a Q-tip was used to moisten the area, the other end was used to wipe it dry. The solvent used was acetone : methanol, 50 : 50 (v/v). Both ends of the Q-tip were cut off and stored in a one-dram vial (Canlab V3010-1). The treated area was removed from the leaf with a 8-mm diameter corkborer and stored in a similar vial. The remaining part of the plant was cut into nine different parts, each wrapped in aluminum foil, and, together with the vials, immediately frozen at -30°C. After all the plants had been harvested, all the samples were freeze-dried for 4

days, and subsequently stored in a desiccator at -30°C.

Extraction

The extraction solvent used was acetone : methanol, 50 : 50 (v/v). The samples were weighed and ground in 25 ml of solvent with a Polytron (Probe generator PT10) at 18,000 rpm for two 1-min periods. After vacuum filtration into a 50-ml boiling flask, the extract was evaporated to dryness in a rotary evaporator (Büch/Brinkman Rotavapor R-110) at 40°C. The filter paper (Whatman No. 2) was saved for further analysis. The residue in the boiling flask was dissolved in 4 ml anhydrous ethyl acetate and transfered to a scintillation vial. The solvent was evaporated in a vacuum desiccator and the vials stored at 2°C.

The efficiency of the extraction procedure was determined by adding a known amount of ^{14}C-chlorsulfuron to non-treated, freeze-dried, Tartary buckwheat plant material at the start of the extraction procedure. The amount of radioactivity lost in the transfer and filtration of the extract, and during the solvent evaporation, was determined.

Quantitative determination

A liquid scintillation counter (Packard Tri-Carb 460CD) was used to determine the amount of radioactivity in each extract. Dissolved in a known volume of anhydrous ethyl acetate, aliquots of the extracts were pipetted into a scintillation vial containing 15 ml of fluor (toluene :

2-ethoxyethanol : PPO : POPOP, 670 : 330: 4 : 1, v/v/w/w)[4]. The vials were counted for 20 min or until the standard error of counting reached 0.2%. The results were automatically adjusted for background and chemiluminescence. The spectral index of the external standard (SIE), coupled with the automatic efficiency control (AEC), was used as the quench indicating parameter (QIP). The quench indicating parameter vs efficiency curve was generated with a series of quenched ^{14}C-toluene standards, using plant extract as the quencher. The counter calculated the sample dpm on the basis of this curve. If the counting efficiency was less than 85%, a smaller aliquot of the plant extract was used.

A biological sample oxidizer (Harvey OX300), using Oxifluor-CO_2 (New England Nuclear) as trapping fluor, combined with the liquid scintillation counter, was used to determine the amount of radioactivity in some of the samples, the Q-tip ends, and in the filter paper discs. Oxidizer efficiency and memory, established and monitored by oxidizing ^{14}C-standards (Polymethylemethacrylate; New England Nuclear) every 50 samples, were 92.9 ± 0.2% (s-) and 0.08 ± 0.02% (s-) respectively. Sample oxidation time was 3 min. Due to differences in counting efficiency between the Oxifluor and the regular fluor, a multiplication factor had to be introduced into the scintillation counter program that calculated the sample dpm.

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[4] PPO - 2,5-diphenyloxazole
POPOP - 1,4-bis-2-(5-phenoxyloxazoyl)-benzene

3.8.2 Mutilation Experiment

In the growth chamber, individual Tartary buckwheat plants were treated with 0 and 7 ug chlorsulfuron (technical), according to the droplet application procedure described in section 3.3. The plants were in the 2-leaf stage and the droplet was applied to the adaxial surface of the second leaf. At different times after treatment, the treated leaves were removed by cutting their petioles 1 cm above the nodes. Fourteen days after treatment, the plants were harvested and the dry weight of the roots and of the shoots was determined.

3.9 Metabolism in Tartary Buckwheat

In the experiment with ^{14}C-chlorsulfuron, the chemical purity of the applied herbicide and the presence of metabolites in the plant extracts were determined by reverse phase thin layer chromatography (RPTLC). An aliquot of the sample, dissolved in 20 ul anhydrous ethyl acetate, was spotted on the plate (Whatman KC18F; Cat. No. 4803-600) in a 10 x 2 mm band and dried with a stream of nitrogen gas. The total amount of radioactivity of a similar aliquot was established by liquid scintillation spectrophotometry as outlined in section 3.8.1.

The chromatography was performed in a saturated tank at 25°C in the dark. Before lowering the plate into the freshly prepared eluant, it was left to equilibrate for 1 hr. The

eluant was acetonitrile (HPLC grade) : 0.1 M phosphate buffer pH 8.0, 60 : 40 (v/v). After the solvent front had travelled 9 cm beyond the origin, the plate was removed from the tank and dried at room temperature for 10 min. A TLC-plate scanner' (Berthold LB2760) located the radioactive regions on the developed plates. These regions were scraped into a liquid scintillation vial, 15 ml of fluor was added, and the samples were counted as outlined in section 3.8.1. The Rf values of ^{14}C-chlorsulfuron and its ^{14}C-metabolite were established by the same procedure.

' Scanning parameters: voltage - 1695 V; time constant - 10 sec; ratemeter - 1K; slit: no. LB6292-1, width - 2 mm, length - 20 mm; counting wire length - 25 mm; scaling factor - 8; scanning speed - 120 mm/hr; gas - P-10 (10% argon, balance methane); gas flow setting - 6-8.

4. RESULTS AND DISCUSSION

4.1 Broadleaved Weed Control

During 1980, preemergence applications of chlorsulfuron were ineffective for the control of Tartary buckwheat in barley (Table II). Only the 40 and 80 g/ha POPI* treatments reduced the Tartary buckwheat biomass significantly, mainly due to a thinning of the stand. However, this level of control is still unacceptable.

The effectiveness of Tartary buckwheat control obtained with postemergence applications of chlorsulfuron in 1980 was dependent upon the rate of application and the leaf stage of the plants (Table II). When applied at the 1-leaf stage, all four rates of chlorsulfuron, 10, 20, 40, and 80 g/ha, resulted in excellent control. The same rates applied at the 2 to 3-leaf stage provided good Tartary buckwheat control also. Although there was no statistically significant difference in Tartary buckwheat biomass between the treatments at the 1- and at the 2 to 3-leaf stage, the level of control at the latter stage was reduced, as indicated by the scoring data. More Tartary buckwheat plants survived at the 2 to 3-leaf stage than at the 1-leaf stage.

On the basis of the Tartary buckwheat biomass, chlorsulfuron applications at the 4 to 6-leaf stage resulted in a similar degree of control as obtained at the 2 to

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* Postplanting-incorporated

Table II. Control of Tartary Buckwheat in Barley.
1980 Experiment - Ellerslie Site.

Treatments†		Rate g/ha	Tartary buckwheat Score	Tartary buckwheat Dry wt. g/m²‡	Barley Yield g/m²‡
			July 28	Aug. 15	Sept. 5
Weedy control			0	218 a	356 b-d
Chlorsulfuron	Pre.E.	10	0	161 a-c	363 b-d
Chlorsulfuron	Pre.E.	20	1	176 a-c	426 a-d
Chlorsulfuron	Pre.E.	40	1	175 a-c	389 b-d
Chlorsulfuron	Pre.E.	80	1	141 a-d	409 a-d
Chlorsulfuron	POPI	10	0	173 a-c	348 c-d
Chlorsulfuron	POPI	20	0	180 a-b	308 d
Chlorsulfuron	POPI	40	1	126 b-d	333 c-d
Chlorsulfuron	POPI	80	0	114 b-e	451 a-d
Chlorsulfuron	1 LS	10	9	3 h	548 a
Chlorsulfuron	1 LS	20	9	0 h	544 a
Chlorsulfuron	1 LS	40	9	1 h	504 ab
Chlorsulfuron	1 LS	80	9	1 h	468 a-c
Chlorsulfuron	2-3 LS	10	5	61 d-h	403 a-d
Chlorsulfuron	2-3 LS	20	6	18 gh	475 a-c
Chlorsulfuron	2-3 LS	40	7	23 f-h	404 a-d
Chlorsulfuron	2-3 LS	80	7	31 e-h	434 a-d
Chlorsulfuron	4-6 LS	10	5	108 b-f	339 c-d
Chlorsulfuron	4-6 LS	20	5	92 c-g	380 b-d
Chlorsulfuron	4-6 LS	40	6	58 d-h	454 a-d
Chlorsulfuron	4-6 LS	80	5	66 d-h	420 a-d
LSD 0.05				73	124

† Leaf stage refers to the number of true leaves of Tartary buckwheat plants at the time of application.
Pre.E. = Preemergence
POPI = Postplanting-incorporated

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

3-leaf stage (Table II). At the 4 to 6-leaf stage, only the 40 and 80 g/ha treatments gave statistically the same reduction in Tartary buckwheat biomass as the treatments at the 1-leaf stage. On the basis of the scoring data, applications of chlorsulfuron at the 4 to 6-leaf stage resulted in only limited control of Tartary buckwheat. Several plants survived the treatments.

Barley yields showed significant increases over weedy control plots in only a few instances. Because these increases were small and the yields were variable, little importance is attached to these differences.

In 1981, postemergence applications of chlorsulfuron provided incomplete control of Tartary buckwheat, regardless of the rate or the time of application (Table III). The level of control of Tartary buckwheat, in terms of biomass at the time of harvest, was statistically the same for all rates of chlorsulfuron tested, both at the 2- and at the 5-leaf stage. The addition of 0.5% Cittowet Plus to the spray solution had no significant effect in the final evaluation. Initially, Cittowet Plus increased the severity of the chlorsulfuron-induced symptons in the Tartary buckwheat plants. However, this increase was only temporary and 3 weeks after the time of application no differences could be observed anymore except for a small reduction in plant height. This reduction was not quantified.

In all except a few instances, wheat yields in treated plots were higher then those on control plots. Because of

Table III. Control of Tartary Buckwheat in Wheat.
1981 Experiment - Ellerslie Site.

Treatments†		Rate	Tartary buckwheat Score	Tartary buckwheat Dry wt. g/m²‡	Wheat Yield g/m²‡
		g/ha	Aug. 1	Aug. 13	Aug. 26
Weedy Control			0	450 a	136 a
Wd. Control + Citt.			0	403 ab	159 a-c
Chlorsulfuron	2 LS	5	3	268 b-e	204 a-d
Chlorsulfuron	2 LS	10	4	284 b-d	260 d-f
Chlorsulfuron	2 LS	20	4	237 c-f	289 ef
Chlors. + Citt.	2 LS	5	5	216 c-f	298 ef
Chlors. + Citt.	2 LS	10	5	354 a-c	324 f
Chlors. + Citt.	2 LS	20	6	260 c-e	297 ef
Chlorsulfuron	5 LS	5	5	205 d-f	227 c-e
Chlorsulfuron	5 LS	10	5	195 d-f	264 d-f
Chlorsulfuron	5 LS	20	6	145 d-f	267 d-f
Chlorsulfuron	5 LS	40	6	167 d-f	264 d-f
Chlorsulfuron	5 LS	60	5	135 d-f	249 d-f
Chlors. + Citt.	5 LS	5	5	225 d-f	145 ab
Chlors. + Citt.	5 LS	10	6	185 d-f	202 a-d
Chlors. + Citt.	5 LS	20	6	122 ef	219 b-e
Chlors. + Citt.	5 LS	40	6	129 ef	251 d-f
Chlors. + Citt.	5 LS	60	7	90 f	258 d-f
LSD 0.05				119	69

† Leaf stage refers to number of true leaves of Tartary buckwheat plants at time of application.
Citt. was Cittowet Plus, applied at 0.5% (v/v) of the spray volume.

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

high variability in these yield data, the importance of the yield effects of herbicide treatments is uncertain.

These results are contrary to what other researchers have reported (30). Traditionally it has been shown that an adjuvant can increase the rate of uptake of a herbicide by plant leaves. Assuming that such is the case with chlorsulfuron also, it still remains to be determined if an increase in the rate of uptake corresponds to an increase in phytotoxicity. The abovementioned increase in inhibition of stem elongation could suggest an increase in the total amount of chlorsulfuron taken up by the Tartary buckwheat plants due to the presence of the adjuvant Cittowet Plus.

The differences in results between the 1980 and the 1981 experiment are enigmatic. Although the crop was barley in 1980 and wheat in 1981, it cannot be assumed that the difference in crop canopies at the time of spraying explains the discrepancy in chlorsulfuron efficacy between these two experiments.

In order to explain differences in herbicide efficacy between different growing seasons or between different experimental sites in the same growing season, the influence of environmental factors must be taken into account (70). The weather data for 1980 and 1981 indicate that, in two of the three instances, higher maximum temperatures at the time of chlorsulfuron application were recorded in 1980 compared to 1981 (Appendix, Table A1). In 1980, the application at the 1-leaf stage was made on the morning of a sunny day,

with the temperatures reaching a maximum of 25°C. The early morning application at the 2 to 3-leaf stage was followed by a 22.8 mm rainfall that began about 3 hr after application. The application at the 4 to 6-leaf stage was made in the evening; the next day was dry and sunny. Compared to the 1981 treatments, most of the 1980 treatments provided excellent control of Tartary buckwheat, except for the lower rates at the later leaf stages, in spite of the differences in weather at the time of application.

In 1981, all applications were made in the evening. In both instances some rainfall was recorded for the following day. In the case of the application at the 5-leaf stage, this following day was very cloudy with a rather high relative humidity. Although the addition of Cittowet Plus to the spray solution resulted in an increase in chlorsulfuron phytotoxicity in the controlled environment experiment (section 4.2.4), assessed 18 days after treatment, this field experiment indicated no significant reduction in Tartary buckwheat biomass at the time of harvest, i.e., 59 and 72 days after treatment. The field observations did indicate an initial increase in the severity of the chlorsulfuron-induced symptoms due to the addition of Cittowet Plus. The controlled environment experiment supports this observation.

It would be presumptuous to conclude that the observed differences in chlorsulfuron efficacy between these two years can be explained in terms of the weather data. These

Table IV. Control of Hempnettle in Barley.
1980 Experiment - Neerlandia Site.

Treatments†	Rate g/ha	Hempnettle Score Aug. 27	Hempnettle Dry wt. g/m²‡ Aug. 27	Barley Yield g/m²‡ Aug. 27
Weedy control		0	113 a	269 ab
Chlorsulfuron	10	9	1 c	324 bc
Chlorsulfuron	30	9	0 c	373 c
Chlorsulfuron	60	9	0 c	307 bc
Bromoxynil/MCPA (1:1)	600	9	3 c	335 bc
Cyanazine/MCPA (1:2)	800	7	12 c	322 bc
Dicamba/MCPA (1:2)	600	7	6 c	328 bc
Linuron/MCPA (1:2)	850	9	1 c	281 bc
Metribuzin	300	9	1 c	286 ab
Metribuzin/MCPA (6:11)	850	9	1 c	231 a
Picloram/2,4-D (1:16)	600	3	46 b	298 b
Dowco 290/2,4-D (1:4)	700	2	62 b	273 ab
Dowco 290/2,4-D (1:4)	900	3	52 b	278 ab
LSD 0.05			29	58

† Treatments were applied when the hempnettle plants had two true leaves.
‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

data do not present sufficient information regarding the micro-environment of the Tartary buckwheat plants before, during, and after herbicide application to validate such conclusions.

The control of hempnettle in barley with chlorsulfuron was excellent (Table IV). Even at the low rate of 10 g/ha, chlorsulfuron provided a level of control comparable to most

Table V. Control of Hempnettle on Fallow.
1980 Experiment - St. Albert Site.

Treatments†	Rate g/ha	Hempnettle Score Aug. 25	Hempnettle Dry wt. g/m²‡ Sept. 2
Weedy control		0	349 a
Chlorsulfuron	5	8	55 c
Chlorsulfuron	10	9	2 c
Chlorsulfuron	25	9	1 c
MCPA amine	500	3	219 b
Chlorsulf. + MCPA am.	5 + 500	8	27 b
LSD 0.05			101

† Treatments were applied when the hempnettle plants had eight true leaves.
‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

recommended herbicides or herbicide mixtures, without any crop damage. The decrease in barley yield in the case of metribuzin and metribuzin/MCPA was due to crop injury. In the case of picloram/2,4-D and of Dowco 290'/2,4-D it was due to poor control of the hempnettle plants.

The control of hempnettle on fallow with chlorsulfuron was good also (Table V). Even though, when compared to the previous experiment, the hempnettle plants were considerably older at the time of spraying, 8- vs 2-leaf stage, low rates of chlorsulfuron gave excellent control. Chlorsulfuron in

'3,6-dichloropicolinic acid

Table VI. Control of Cleavers in Barley.
1980 Experiment - Villeneuve Site.

Treatments†	Rate g/ha	Cleavers Score Aug. 29	Cleavers # Plants per m²‡ Aug. 29	Barley Yield g/m²‡ Aug. 29
Weedy control		0	19 a	524 cd
Chlorsulfuron	10	8	6 b-e	484 b-d
Chlorsulfuron	30	9	1 de	504 b-d
Chlorsulfuron	60	8	1 e	557 d
Bromoxynil/MCPA (1:1)	600	8	2 de	539 cd
Cyanazine/MCPA (1:2)	800	8	4 c-e	488 b-d
Dicamba/MCPA (1:2)	600	9	1 e	510 b-d
Linuron/MCPA (1:2)	850	7	7 b-e	426 b
Metribuzin	300	7	11 bc	468 b-d
Metribuzin/MCPA (6:11)	850	6	10 b-d	458 bc
Picloram/2,4-D (1:16)	600	8	1 e	500 b-d
Dowco 290/2,4-D (1:4)	700	6	13 ab	483 b-d
Dowco 290/2,4-D (1:4)	900	7	12 ab	479 b-d
LSD 0.05			7	78

† Treatments were applied when the cleavers had two whorls of leaves.
‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

combination with MCPA amine provided good control as well.

Chlorsulfuron has proved to be an effective herbicide for the control of cleavers in barley (Table VI). Rates of 30 and 60 g/ha, applied when the cleavers had two whorls of leaves, provided excellent control.

4.2 Influence on the Growth of Tartary Buckwheat

4.2.1 Growth Rate

The rate of growth of Tartary buckwheat plants was significantly reduced after they had been sprayed with chlorsulfuron 20 g/ha when in the 3-leaf stage (Figures[10] 2 and 3). Up to 15 days after application, chlorsulfuron-treated plants did not gain in height, but they did increase in biomass, albeit at a lower rate than the control plants. This is due to an inhibition of elongation of the main stem, and a continued development of new leaves, especially axillary leaves, and reproductive structures. The result is a short, bushy plant (Figure 4).

At 15 days after treatment, a great diversity in symptoms became apparent. In some of the treated plants, the flower stalks and the main stem began to elongate, resulting in an increase in the average plant height. In other treated plants, many of the leaves became necrotic, resulting in a loss of biomass. Thus it appears that, even though the initial symptoms are rather similar for all plants, some plants are able to survive and gain in height, while others become necrotic and slowly die.

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[10] The vertical bars on these and subsequent graphs represent the variations of the means on the basis of plus or minus two times their standard error.

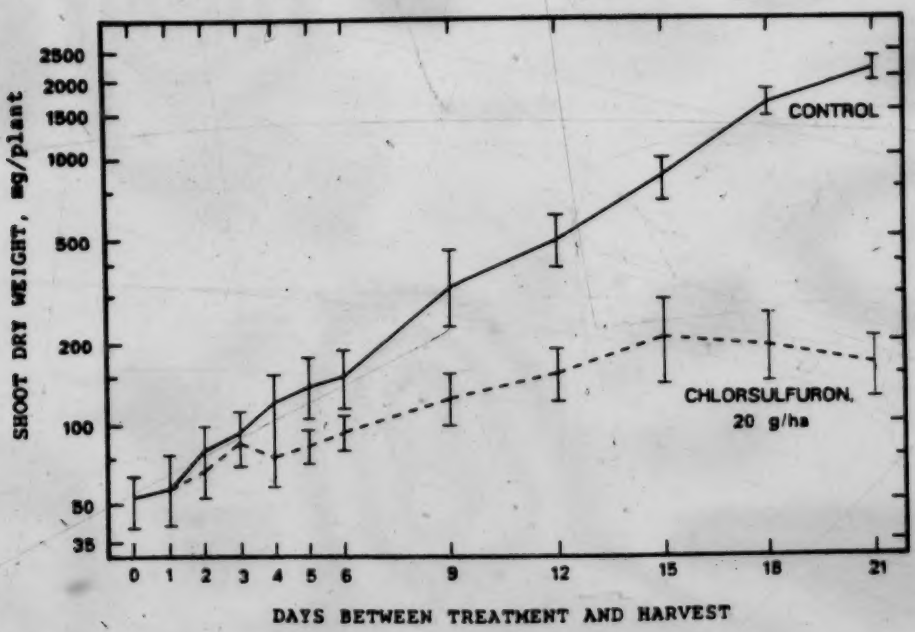

Figure 2. Effect of Chlorsulfuron on the Dry Weight of Tartary Buckwheat Plants During a 21-Day Period Following Treatment at the 3-Leaf Stage.

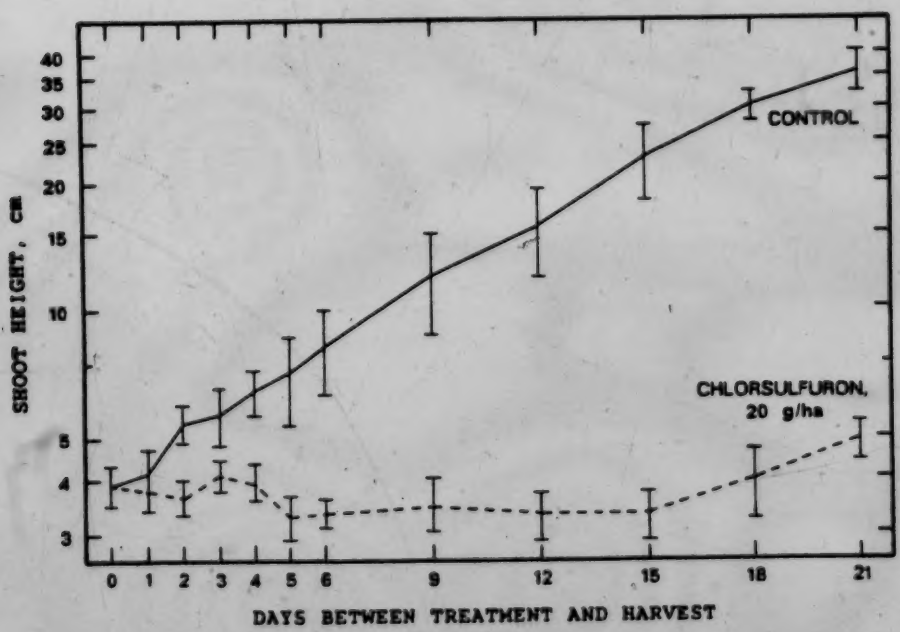

Figure 3. Effect of Chlorsulfuron on the Height of Tartary Buckwheat Plants During a 21-Day Period Following Treatment at the 3-Leaf Stage.

Figure 4. Inhibition of Stem Elongation of Tartary Buckwheat Plant Following Treatment with Chlorsulfuron.

Figure 5. Stem Shriveling of Tartary Buckwheat Plant Following Treatment with Chlorsulfuron.

4.2.2 Symptoms

Number of leaves

When chlorsulfuron, 0.5 to 40 ug/plant, was applied in a 10-ul droplet to Tartary buckwheat plants that had one fully emerged true leaf (1-leaf stage), the development of new leaves was slowed down considerably after one day. At 20 and 40 ug/plant, a complete arrest of new leaf development occurred after 3 days. Near the end of the 10-day observation period, small new leaves began to develop on those plants that had received less than 20 ug/plant, especially on those that had received only 0.5 ug/plant.

The application of chlorsulfuron to Tartary buckwheat plants that had two fully emerged true leaves (2-leaf stage), resulted in a reduction in the rate of new leaf development. This reduction increased as the dosage increased. However, even the highest dosage, 40 ug/plant, did not result in a complete arrest of new leaf development.

A similar observation was made on those Tartary buckwheat plants that were treated when they had three fully emerged true leaves (3-leaf stage). The rate of new leaf development was also reduced. In this case, this reduction was less dependent upon the amount of chlorsulfuron per plant than at the 1- and 2-leaf stage. The rate of development of new leaves on Tartary buckwheat plants that had four fully emerged true leaves (4-leaf stage) at the time of treatment, was also slightly reduced. No dosage dependency could be detected.

Axillary leaves

The development of axillary leaves was completely inhibited by dosages greater than 0.5 ug/plant, when applied at the 1-leaf stage. At the 0.5 ug/plant dosage, a few small axillary leaves were observed 7 days after treatment. Control plants began to develop axillary leaves 3 to 5 days after treatment. When chlorsulfuron was applied at the 2-leaf stage, limited axillary leaf development occurred only at dosages of 0.5 to 5 ug/plant. These leaves were very small. At higher dosages, axillary leaf development occurred only sporadically, and usually near the end of the 10-day observation period.

None of the dosages of chlorsulfuron applied at the 3-leaf stage resulted in the complete inhibition of axillary leaf development. All chlorsulfuron-treated plants developed axillary leaves that were considerably smaller, and fewer in number, than the ones of the control plants. Application of chlorsulfuron at the 4-leaf stage of Tartary buckwheat did not inhibit axillary leaf development either. Although these axillary leaves were smaller, and fewer in number, than the ones of the control plants, they were larger, and greater in number, than the axillary leaves that developed following treatment at the 3-leaf stage.

Chlorosis

Chlorosis of the apex is one of the first visually observable symptoms in Tartary buckwheat plants induced by treatment with chlorsulfuron. At all four leaf stages

tested, and at all dosages of chlorsulfuron, 0.5 to 40 ug/plant, chlorosis of the newly formed tissue at the apex was observed one day after treatment. The severity of the apical chlorosis was similar for the plants treated at the 1-, 2-, or 3-leaf stage, regardless of chlorsulfuron dosage. Apical chlorosis of plants treated at the 4-leaf stage was less severe.

Except for the plants treated at the 4-leaf stage, the duration of the chlorosis was dependent upon the chlorsulfuron dosage. Plants that had been treated at the 1-leaf stage and that had received more than 2 ug of chlorsulfuron, remained chlorotic until harvest, 14 days after treatment. The apices of the plants that had received lower dosages, gradually became greener after about 5 days. The apical chlorosis of plants treated at the 2- and 3-leaf stages decreased at 5 days after treatment, regardless of chlorsulfuron dosage.

At 3 days after treatment, the non-apical parts of the Tartary buckwheat plants treated at the 1-, 2-, and 3-leaf stage became slightly chlorotic. This chlorosis remained until harvest time, 14 days after treatment and it was independent of chlorsulfuron dosage. No non-apical chlorosis was observed in plants treated at the 4-leaf stage.

Stem shriveling

The shriveling of the stem immediately below the cotyledonary node is a typical, chlorsulfuron-induced, symptom in Tartary buckwheat plants (Figure 5). The extent

and the severity of this shriveling was a function of the leaf stage of the plants at the time of treatment and the length of time after treatment. The onset of stem shriveling could be observed 4 days after treatment.

Stem shriveling was most severe in plants treated at the 1- and 2-leaf stage. In many instances, the complete stem section between the cotelydonary node and the roots suffered from shriveling to a greater or lesser degree, regardless of the chlorsulfuron dosage. The Tartary buckwheat plants that had been treated with chlorsulfuron when they were in the 3-leaf stage, had considerably less stem shriveling than the ones treated in the 1- and 2-leaf stage. No stem shriveling was induced in plants treated at the 4-leaf stage.

Reproductive structures

Plants that had been treated with chlorsulfuron at the 1-leaf stage, did not develop any reproductive structures, except at the lowest dosage, i.e., 0.5 ug/plant. The higher dosages completely prevented the formation of reproductive structures at this leaf stage. A few reproductive structures were observed in plants that had been treated with chlorsulfuron at the 2-leaf stage, using dosages greater than 10 ug/plant. At lower dosages, the reproductive structures were more abundant. However, their development was delayed considerably compared to the control plants.

Regardless of the chlorsulfuron dosage, plants treated at the 3- and 4-leaf stage developed reproductive

structures. Although the development of these structures was slower than in the control plants, other experiments have indicated that viable seeds will be produced.

4.2.3 Dry Weight 14 Days after Treatment

The Tartary buckwheat plants used to observe the different symptoms induced by treatment with several dosages of chlorsulfuron, were harvested 14 days after treatment. The treatment of Tartary buckwheat with chlorsulfuron at the 1-, 2-, or 3-leaf stage, resulted in a flat dosage vs response curve for all dosages between 0.5 and 40 ug/plant, both in terms of shoot and of root dry weight. Applied at the 4-leaf stage, a dosage of 0.5 ug/plant was less effective in reducing the shoot dry weight than dosages of 2 to 40 ug/plant. At this leaf stage, no differences between the different dosages in terms of root dry weight reduction were observed (Appendix, Figures A1 and A2).

In order to compare the effectiveness of chlorsulfuron at the four different leaf stages, the increase in shoot dry weight of the treated plants was expressed as a percentage of the increase in the shoot dry weight of the control plants during the 14-day period between treatment and harvest. In Figure 6, these percentages are related to the applied dosages of chlorsulfuron. These results confirm the observations that the efficacy of chlorsulfuron is significantly dependent upon the leaf stage of the Tartary buckwheat plant at the time of treatment. The efficacy

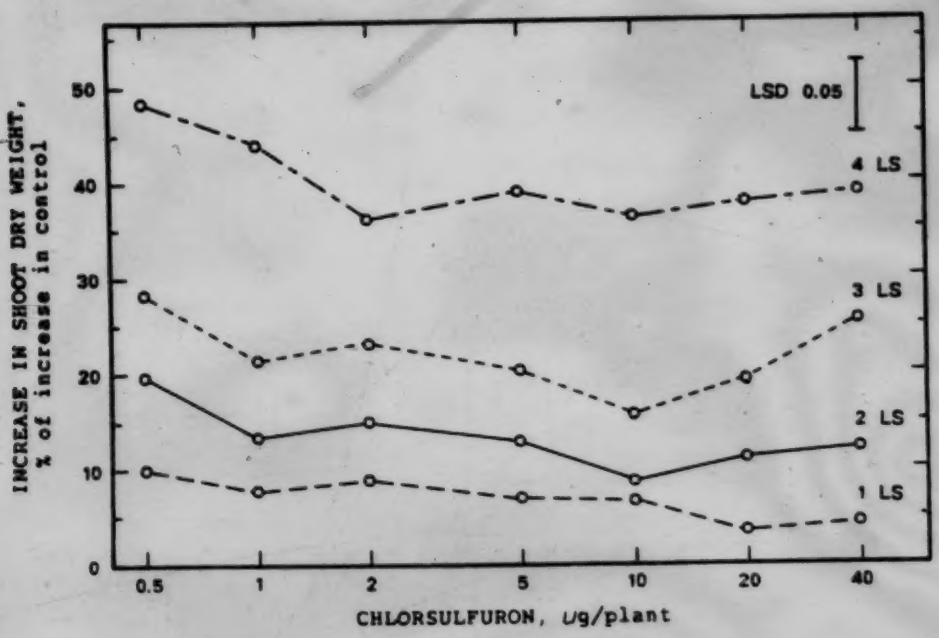

Figure 6. Effect of Increasing Dosages of Chlorsulfuron, in Single-Droplet Application at Four Different Leaf Stages, on the Increase in Shoot Dry Weight of Tartary Buckwheat Plants, Harvested 14 Days after Treatment.
Chlorsulfuron dosages are plotted on logarithmic scale.
LSD value is between dosages of chlorsulfuron at the same leaf stage.

decreases as the number of leaves at the the time of application increases from one to four. Because the root dry weight data were highly variable, no additional information regarding chlorsulfuron efficacy was obtained when the increase in root dry weight of the chlorsulfuron-treated plants was expressed in terms of the increase in root dry weight of the control plants during the 14-day period after treatment.

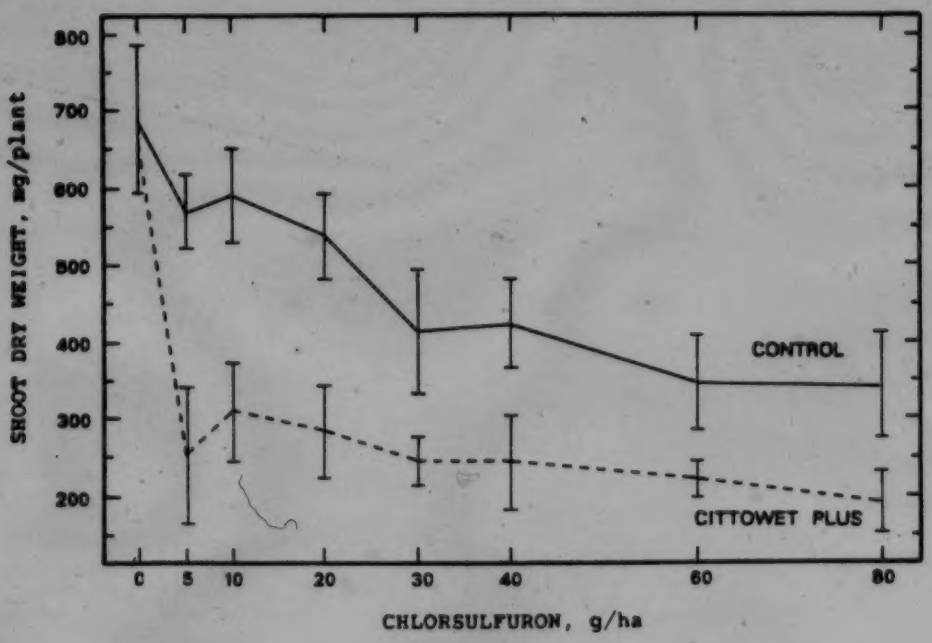

Figure 7. Effect of a Spray Application of Chlorsulfuron, with or without Cittowet Plus (0.5% v/v), on the Shoot Dry Weight of Tartary Buckwheat Plants, 18 Days after Treatment at the 4-Leaf Stage.

4.2.4 Influence of Adjuvant

The influence of the adjuvant Cittowet Plus on the efficacy of chlorsulfuron is represented in Figure 7 in terms of dosage vs response curves. The spray application was made when the Tartary buckwheat plants were in the 4-leaf stage. At all rates of chlorsulfuron tested, the addition of 0.5% Cittowet Plus to the spray solution significantly decreased the dry weight at the time of harvest. This decrease is primarily due to the formation of fewer and smaller axillary leaves and the inhibition of stem elongation.

Without Cittowet Plus, the curve consisted of two sections, a 5 to 20 g/ha section and a 30 to 80 g/ha section. Within each section, no statistically significant differences were observed. With the adjuvant, no statistically significant difference between any of the rates of chlorsulfuron could be observed.

4.2.5 Interception of Spray Solution

The amount of chlorsulfuron intercepted by Tartary buckwheat plants during a spray application of 20 g/ha, is recorded in Table VII. Plants that were in the 4-leaf stage at the time of spraying intercepted more herbicide than those that were in the 2-leaf stage, due to their larger total leaf area. When the amount of chlorsulfuron intercepted is expressed in terms of ug/g shoot dry weight, the plants sprayed in the 2-leaf stage attained a higher average concentration of chlorsulfuron than the ones sprayed in the 4-leaf stage. Presumably this is due to a relatively greater increase in the dry weight of Tartary buckwheat plants than in the leaf area that can intercept the spray solution. The difference in chlorsulfuron interception (ug/gr dry weight) between the 2- and 4-leaf stage parallels the observed difference in chlorsulfuron efficacy between these leaf stages as observed in the 1980 field experiment.

Table VII. Interception of Chlorsulfuron by Tartary Buckwheat Plants, Sprayed at 20 g/ha.

Leaf stage	ug/plant†	ug/g dry wt.†
2	5.15 (0.24)	84.9 (3.9)
4	9.30 (0.34)	66.8 (3.5)

† Mean and standard error on the basis of ten replicates.

4.2.6 Discussion

On the basis of these results it appears that the susceptibility of Tartary buckwheat plants to chlorsulfuron decreases as the leaf stage at the time of application advances from one to four. This decrease in susceptibility manifests itself in the failure of chlorsulfuron to inhibit the development of new leaves and of reproductive structures when it is applied at the later leaf stages. Only a delay in the development of these plant parts has been observed. It also appears that the effect of increasing the dosage of chlorsulfuron was minimal, and restricted primarily to an increase in the length of the delay in development.

The interpretation of these morphological responses must take into account the stage of development of the plant parts at the time of herbicide application. It appears that, unless the development of new leaves and of reproductive structures is affected at an early differentiation stage, chlorsulfuron is unable to exert a lasting effect. However, chlorsulfuron is able to prevent the elongation of the main

stem, regardless of the development stage at the time of application. The steps involved in this inhibition process have not been investigated. Interaction of chlorsulfuron with the plant hormones must not be excluded from investigations into the mode of action of this herbicide.

The observed shriveling of the Tartary buckwheat stem below the cotyledonary node, decreasing in severity as the leaf stage at the time of application advanced from one to three, is a symptom unique to this species. The nature of this shriveling and its subsequent effect on the growth of the plants have not been investigated. Even though this study does not supply any information regarding a possible interference with the translocation of assimilates to the roots due to a disruption of the phloem tissue in the shriveled stem section, such a possibility must not be disregarded.

On the basis of these experiments, the chlorosis of the apex, and subsequently of the non-apical parts, following treatment with chlorsulfuron, cannot be elucidated further. The chlorosis-associated reduction in the net photosynthetic rate could contribute to the observed reduction and/or delay in the growth and development of the Tartary buckwheat plants.

Caution must be excercised when the effect on the growth of plants that received a particular dosage of chlorsulfuron in the 10-μl single-droplet form, is related to the effect on the growth of similar plants that have been

sprayed with a corresponding solution of chlorsulfuron, on the basis of the spray solution interception study. Any comparison must take into account such factors as (i) the amount of chlorsulfuron at the site of application and its effect on absorption and translocation, (ii) the ability of a plant to metabolize or inactivate the herbicide as it relates to its distribution in the plant, and (iii) the distance between the site of application and the site of action. If we want to relate single-droplet applications, made in the controlled environment, to field applications, the influence of the different plant micro-environments also must be taken into account.

4.3 Interaction with Wild Oat Herbicides

The interaction between chlorsulfuron and wild oat herbicides can be evaluated in terms of the effect of the different wild oat herbicides on the efficacy of chlorsulfuron and vice versa. The former effect received only marginal attention in this study. It was observed that the control of Tartary buckwheat by chlorsulfuron was enhanced when a postemergence wild oat herbicide was present in the spray solution, especially in the 1981 experiment (Table X). The phytotoxic symptoms were more severe and fewer plants survived. No evaluation in terms of Tartary buckwheat biomass at the time of harvest was performed. Neither was it established whether the observed effect was due to the active ingredients of the wild oat herbicides or to the adjuvants present in the wild oat herbicide formulations.

In both years, chlorsulfuron did not reduce the efficacy of the wild oat herbicide difenzoquat (1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium methyl sulfate), even though the level of wild oat control achieved was different in the two years (Tables VIII and X). In 1980, the control of wild oats with difenzoquat was excellent. The addition of 10 or 40 g/ha of chlorsulfuron in a tank mixture did not reduce this. Wild oat control in 1981 was poor, and remained poor after adding 20 g/ha of chlorsulfuron. Although it has been reported that the efficacy of difenzoquat is leaf-stage-dependent (36), and increases as

Table VIII. Control of Wild Oats in Barley with Tank Mixtures of Chlorsulfuron and Wild Oat Herbicides. 1980 Experiment - Ellerslie Site.

Treatments†	Rate kg/ha	Wild oats		Barley
		Culms per m²‡ Sept. 9	Dry wt. g/m²‡ Sept. 9	Yield g/m²‡ Sept. 9
Weedy control		485 a	432 a	356 a
Diclofop methyl	0.70	1 e	1 c	601 e
Difenzoquat	0.85	0 e	0 c	551 c-e
Barban	0.35	150 de	118 bc	490 a-e
Triallate (POPI)	1.40	139 de	148 bc	471 a-e
Chlorsulfuron	0.01	432 a-c	391 a	408 a-c
Chlorsulfuron	0.04	423 a-c	502 a	356 a
Chlors. (POPI)	0.01	311 a-d	401 a	377 ab
Chlors. (POPI)	0.04	462 ab	484 a	358 a
Chlors. + DCM	0.01+0.70	6 e	4 c	537 c-e
Chlors. + DCM	0.04+0.70	20 e	16 bc	583 de
Chlors. + Dif.	0.01+0.85	3 e	1 c	584 de
Chlors. + Dif.	0.04+0.85	1 e	1 c	598 de
Chlors. + Barban	0.01+0.35	230 b-e	155 bc	531 b-e
Chlors. + Barban	0.04+0.35	193 c-e	194 b	493 a-e
Chlors. + Triall.	0.01+1.40	147 de	164 bc	455 a-e
Chlors. + Triall.	0.04+1.40	119 de	120 bc	443 a-d
LSD 0.05		222	163	130

† Treatments were applied at the wild oat leaf stage appropriate for the wild oat herbicide.
POPI = Postplanting-incorporated

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

the wild oat plants advance from the 3- to the 5-leaf stage, this does not account for the discrepancy in efficacies during these two years. In both years the majority of the wild oat plants were in the 4 to 5-leaf stage at the time of spraying.

The control of wild oats by flamprop methyl {methyl (±)-2-[N-(3-chloro-4-fluorophenyl)benzamido]propionate}, was not affected by the addition of chlorsulfuron. Flamprop methyl (FPM) at 0.50 kg/ha in 1980 and 0.50 or 0.60 kg/ha in 1981 resulted in good wild oat control. The addition of 10 or 40 g/ha in 1980 and 20 gram/ha in 1981 did not significantly reduce its efficacy (Tables IX and X).

Diclofop methyl (DCM) at 0.70 kg/ha gave excellent control of wild oats in 1980. The addition of 10 or 40 g/ha of chlorsulfuron resulted in the survival of a few more wild oat plants. However, there is no statistical basis to suggest that chlorsulfuron antagonizes the action of diclofop methyl (Table VIII). In 1981, both the 0.70 and the 0.85 kg/ha rate provided excellent wild oat control. The addition of 20 g/ha of chlorsulfuron did not decrease this level of control (Table X). In a mixture, at the recommended rates, chlorsulfuron and diclofop methyl appear to be fully compatible. A similar observation can be made for the mixture of chlorsulfuron with HOE 00654, the active isomer of diclofop methyl (Table X).

The control of wild oats with barban (4-chloro-2-butynyl *m*-chlorocarbanilate) or a mixture of

Table IX. Control of Wild Oats in Wheat with Tank Mixtures of Chlorsulfuron and Flamprop Methyl. 1980 Experiment - Ellerslie Site

Treatments†	Rate kg/ha	Wild oats		Wheat
		Culms per m²‡ Sept.9	Dry wt. g/m²‡ Sept.9	Yield g/m²‡ Sept.9
Weedy control		448 a	447 a	228 a
Chlorsulfuron	0.01	449 a	536 a	194 a
Chlorsulfuron	0.04	352 a	455 a	194 a
Flamprop methyl	0.50	3 b	2 b	478 cd
Chlorsulf. + FPM	0.01+0.50	27 b	22 b	450 bc
Chlorsulf. + FPM	0.04+0.50	28 b	26 b	376 bc
LSD 0.05		102	101	70

† Applications were made when the wild oat plants had three leaves.

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

barban and flamprop methyl was poor during both years (Tables VIII and X). Although the addition of 10 or 40 g/ha of chlorsulfuron to the barban treatment caused an increase in the number of surviving wild oat plants in 1980, the difference was not significant. No such increase was observed in 1981.

The efficacy of barban is greatly dependent upon the leaf stage of the wild oats at the time of application. Under normal growing conditions, the optimum leaf stage of wild oats for control by barban is the 1 to 3-leaf stage.

Table X. Control of Wild Oats in Wheat with Tank Mixtures of Chlorsulfuron and Wild Oat Herbicides. 1981 Experiment - Ellerslie Site.

Treatments†	Rate kg/ha	Wild oats Dry wt. g/m²‡ Aug. 26		Wheat Yield g/m²‡ Aug. 26	
Weedy control		368	a	189	a
Difenzoquat	0.85	152	c	220	ab
Diclofop methyl	0.70	8	d	277	bcde
Diclofop methyl	0.85	2	d	287	bcde
HOE 00654	0.35	21	d	306	de
Flamprop methyl	0.50	49	d	293	cde
Flamprop methyl	0.60	12	d	321	e
Barban + FPM	0.15+0.25	175	c	245	abcd
Barban	0.35	285	b	225	abc
Dif.+ Chlorsulfuron	0.85+0.02	151	c	257	bcde
DCM + Chlorsulfuron	0.70+0.02	6	d	266	bcde
DCM + Chlorsulfuron	0.85+0.02	5	d	288	bcde
HOE 00654 + Chlorsulf.	0.35+0.02	25	d	288	bcde
FPM + Chlorsulfuron	0.50+0.02	53	d	258	bcde
FPM + Chlorsulfuron	0.60+0.02	42	d	249	abcd
Barb.+FPM+Chlorsulf.	0.15+0.25+0.02	154	c	271	bcde
Barban + Chlorsulf.	0.35 +0.02	239	bc	254	bcde
LSD 0.05		80		57	

† Treatments were applied at the wild oat leaf stage appropriate for the wild oat herbicide.

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

Under dry growing conditions, the 1.5 to 3.5-leaf stage becomes the optimum, while under wet growing conditions the optimum is found at the 1.5 to 2.5-leaf stage of the wild oats (35). The poor wild oat control by barban obtained in this study cannot be attributed to the time of application; in both years all barban treatments were applied at the 2 to 2.5-leaf stage.

The combination of chlorsulfuron with triallate, a preemergence, soil-incorporated wild oat herbicide, was tested in the 1980 experiment (Table VIII). Chlorsulfuron did not influence the efficacy of triallate. Because chlorsulfuron showed more promise as a postemergence herbicide than as a preemergence one (Table II), the combination with triallate was not repeated in 1981.

The results of the field experiments were reinforced by the data obtained in a greenhouse experiment (Table XI). Although the levels of wild oat control, expressed in terms of the dry weight of wild oats 40 days after treatment, varied between the four postemergence wild oat herbicides tested, none of them was significantly influenced by the addition of 20, 40, or 80 g/ha of chlorsulfuron. In the case of difenzoquat and flamprop methyl, the growth of the wild oat plants was inhibited following the herbicide applications. At the time of harvest these wild oat plants were chlorotic and lacked tillers. Chlorosis was more extensive in those plants that had not received any chlorsulfuron than in those that had. In the case of barban

Table XI. **Effect of Chlorsulfuron on Wild Oat Herbicide Efficacy. Greenhouse Experiment.**

Wild oat herbicides†	Rate kg/ha	Dry weight wild oats, g/pot — Chlorsulfuron, g/ha			
		0	20	40	80
Barban	0.35	527	467	511	549
Difenzoquat	0.85	137	158	158	172
Diclofop methyl	0.70	504	498	616	649
Flamprop methyl	0.50	134	131	120	139
Benzoylprop ethyl	1.40	192	360	319	300
LSD 0.05			177		

† Applications were made at the leaf stage appropriate for the wild oat herbicide. Wild oat herbicides were applied as tank mixtures with chlorsulfuron.

and diclofop methyl, growth of the wild oat plants was initially inhibited following the herbicide treatments. This initial inhibition was overcome after 7 to 10 days and tillering began at this time. At the time of harvest the plants were only slightly chlorotic. Diclofop methyl-treated plants that had received chlorsulfuron were greener than those that had not received any chlorsulfuron.

Diclofop methyl did not perform as well in the greenhouse experiment as it did in the field experiments. Dortenzio et al. (24) reported that the efficacy of diclofop methyl can be influenced by the soil moisture conditions at

the time of application. Low moisture conditions, 2 to 3% above wilting point, resulted in a 15 to 50% loss of herbicide activity. High soil moisture conditions, 67% of field capacity or more for 2 to 4 days following application, were needed to achieve maximum efficacy. However, these observations do not account for the lower diclofop methyl efficacy obtained in the greenhouse experiment, since soil moisture in the pots was maintained at 60 to 80% of field capacity.

Both the field experiments and the greenhouse experiment indicate that the influence of chlorsulfuron on the efficacy of postemergence wild oat herbicides is minimal. No significant differences in terms of wild oat biomass at the time of harvest could be observed. Chlorsulfuron seemed to reduce the chlorosis induced by difenzoquat, flamprop methyl, and diclofop methyl.

4.4 Tolerance of Cereals

Foliar applications of chlorsulfuron, ranging from 5 to 80 g/ha, some with and some without adjuvant, did not cause any significant injury to either wheat or barley, as evaluated in the different field experiments.

In the greenhouse experiment, designed to evaluate the effect of normal and high rates of chlorsulfuron on wheat, barley, and oats, a large amount of variability was encountered. In spite of this, and although the statistical analysis did not take into account the change in competition due to removal of one plant from each pot at the different harvest times, some general trends could be observed.

In this experiment, chlorsulfuron (20 to 320 g/ha), applied at the 3-leaf stage, did not inhibit the development of new leaves or the formation of tillers. All chlorsulfuron-treated plants attained full maturity at the same time as the control plants, and all produced viable seeds. In general, no obvious, visual deformities of any of the plant parts were observed. Although the barley plants were slightly chlorotic for a 7 to 9-day period following the application, no such symptom was observed in either oat or wheat. In barley, the highest rate of chlorsulfuron, 320 g/ha, did cause some wrinkling of the blade of the fourth leaf. Presumably this is due to mechanical hindrance encountered during the emergence of this particular leaf.

Figure 8 demonstrates the effect of chlorsulfuron on the dry weight of oat plants at four different times after

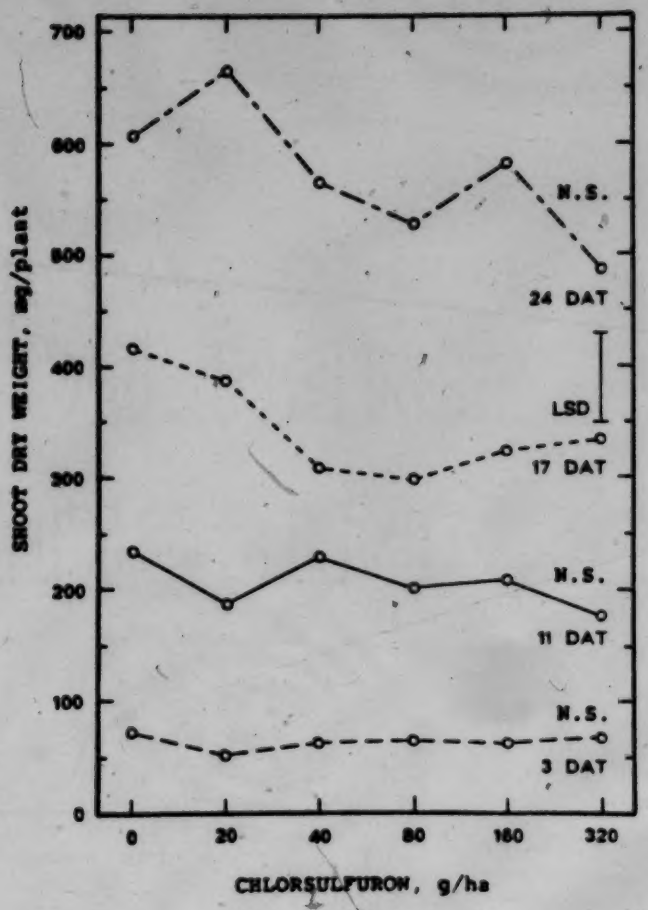

Figure 8. Influence of Increasing Rates of Chlorsulfuron on the Shoot Dry Weight of Oat Plants 3, 11, 17, and 24 Days after Treatment. Chlorsulfuron rates are plotted on logarithmic scale. LSD bar represents value at P = 0.05. N.S. indicates the F-value was not significant at P = 0.05.

treatment. Significant differences can be observed at 17 days after treatment. A similar trend can be discerned in Figures 9 and 10, representing the influence on the dry weight of wheat and barley, respectively. In the case of barley, the differences become significant only at 24 days

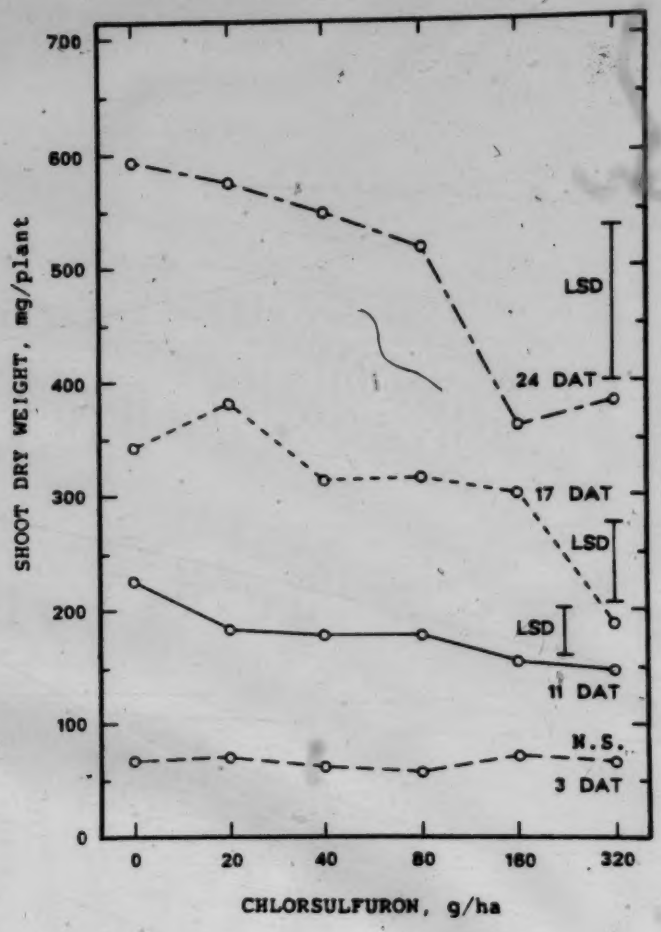

Figure 9. Influence of Increasing Rates of Chlorsulfuron on the Shoot Dry Weight of Wheat Plants 3, 11, 17, and 24 Days after Treatment. Chlorsulfuron rates are plotted on logarithmic scale. LSD bars represent values at P = 0.05. N.S. indicates the F-value was not significant at P = 0.05.

after treatment, while in the case of wheat significant differences can be observed already 11 days after treatment. In all instances the reduction in dry weight can be attributed to a reduction in plant height, which was mainly due to an inhibition of elongation of the internode. Figures

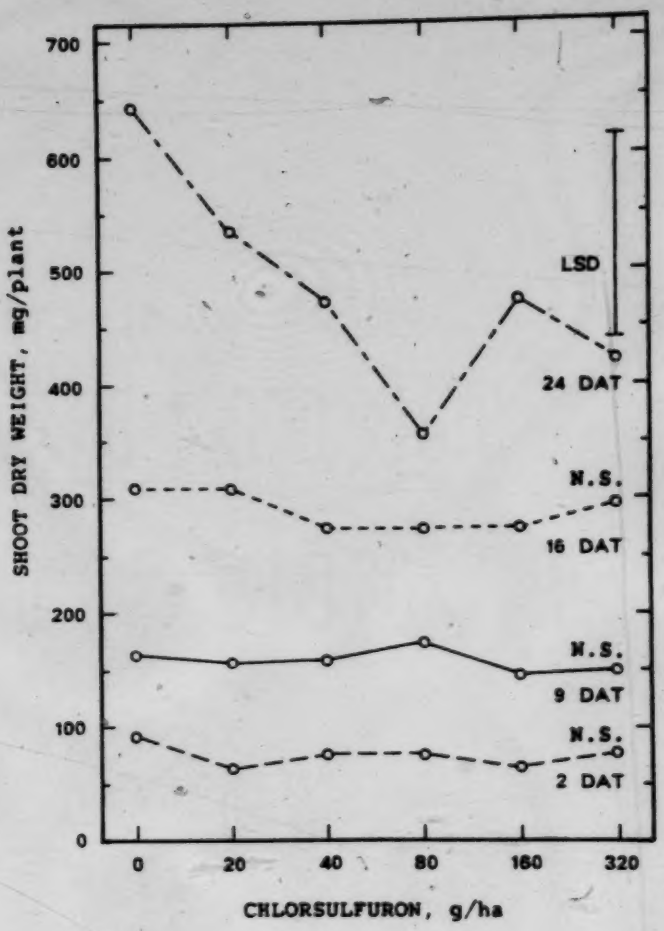

Figure 10. Influence of Increasing Rates of Chlorsulfuron on the Shoot Dry Weight of Barley Plants 2, 9, 16, and 24 Days after Treatment. Chlorsulfuron rates are plotted on logarithmic scale. LSD bar represents value at P = 0.05. N.S. indicates the F-value was not significant at P = 0.05.

11 and 12 illustrate this symptom.

On the basis of this greenhouse experiment it can be concluded that low rates of chlorsulfuron (20 and 40 g/ha) have no visible effect on the growth of wheat (cv Neepawa) or oats (cv Cascade). In the case of barley (cv Galt), a

reduction in dry weight of the plants was observed at these low rates. Even though higher rates did reduce the dry weight of the wheat, barley, and oat plants, no delay in maturity was observed. The field experiments confirmed the observations for wheat, but not for barley. No injury to the barley crop was observed in any of the field experiments. Because none of the field experiments with chlorsulfuron included a weed-free treatment, no data regarding the effect of chlorsulfuron on the grain yield of wheat and barley can be submitted.

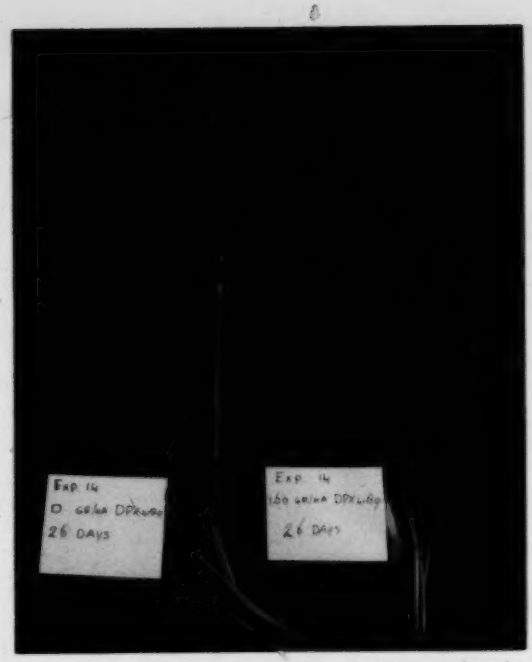

Figure 11. Effect of Chlorsulfuron (160 g/ha) on Barley, 26 Days after Treatment.

Figure 12. Effect of Chlorsulfuron (160 g/ha) on Barley, 37 Days after Treatment.

4.5 Carryover in Soil

4.5.1 Effect on Rapeseed Crop

When, in the spring of 1980, chlorsulfuron was used to control Tartary buckwheat in barley, the amounts that remained in the soil were sufficient to affect the growth of rapeseed in 1981. The chlorsulfuron present in the soil affected both the emergence and the growth of rapeseed. Emergence on the plots that had received 40 and 80 g/ha in 1980 was 15 to 25% less than on control plots. On the plots that had received 80 g/ha in 1980, 30 to 50% of the rapeseed plants died soon after emergence.

The growth of rapeseed on plots that had received chlorsulfuron in 1980 was slow, resulting in a 10 to 30% shorter stand and a slight delay in maturity. The dry weight data indicate there was no significant effect on the biomass of rapeseed at the 10 and 20 g/ha rates of chlorsulfuron, regardless of the method or the time of application (Table XII). The 40 g/ha rate significantly reduced the biomass of rapeseed in the POPI treatment, while no significant effect was observed in the other treatments. Presumably this was due to a higher concentration of chlorsulfuron in the soil. This could have been caused by the absence of a crop canopy at the time of application, and by a reduced rate of loss by means of photodegradation, due to incorporation into the soil. However, the chlorsulfuron concentration in the soil, determined by means of the corn root bioassay (section

Table XII. Effect of One-Year Chlorsulfuron Carryover on the Biomass of Rapeseed. Ellerslie Site.

Treatments†		Rate g/ha	Spray date 1980	Rapeseed - 1981 Score July 3	Dry wt. g/m²‡ Aug. 7
Control				9	353 a-c
Chlorsulfuron	Pre.E.	10	June 12	8	383 ab
Chlorsulfuron	Pre.E.	20	June 12	8	306 b-e
Chlorsulfuron	Pre.E.	40	June 12	7	389 ab
Chlorsulfuron	Pre.E.	80	June 12	5	237 d-f
Chlorsulfuron	POPI	10	June 12	8	380 ab
Chlorsulfuron	POPI	20	June 12	8	335 a-d
Chlorsulfuron	POPI	40	June 12	6	239 d-f
Chlorsulfuron	POPI	80	June 12	5	187 f
Chlorsulfuron	1 LS	10	June 20	9	352 a-c
Chlorsulfuron	1 LS	20	June 20	8	365 a-b
Chlorsulfuron	1 LS	40	June 20	6	331 a-d
Chlorsulfuron	1 LS	80	June 20	4	242 d-f
Chlorsulfuron	2-3 LS	10	July 3	8	432 a
Chlorsulfuron	2-3 LS	20	July 3	7	282 b-f
Chlorsulfuron	2-3 LS	40	July 3	5	291 b-f
Chlorsulfuron	2-3 LS	80	July 3	2	44 g
Chlorsulfuron	4-6 LS	10	July 7	8	330 a-d
Chlorsulfuron	4-6 LS	20	July 7	8	338 a-d
Chlorsulfuron	4-6 LS	40	July 7	6	254 c-f
Chlorsulfuron	4-6 LS	80	July 7	3	207 ef
LSD 0.05					93

† Leaf stage refers to the number of true leaves of Tartary buckwheat at treatment time.
Pre.E. = Preemergence
POPI = Postplanting-incorporated

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

4.5.2), does not support this hypothesis.

Chlorsulfuron, applied at a rate of 80 g/ha, significantly reduced the biomass of rapeseed in all instances. This reduction was greatest following application at the 2 to 3-leaf stage. The application of chlorsulfuron at this leaf stage of the Tartary buckwheat in 1980 was followed by a 22.8 mm rainfall within 24 hours (Appendix, Table A1). The hypothesis is that the rainwater has carried the chlorsulfuron into the soil, thus reducing the potential loss due to photodegradation. The soil of these plots was not analyzed by means of the corn root bioassay.

The effect on the growth of rapeseed of both a one-year and a two-year carryover of chlorsulfuron is reported in Table XIII. In 1980, the one-year carryover caused a thinning of the rapeseed stand and a delay in emergence and in attaining maturity. The observed thinning, ranging from approximately 30 to 65%, increased with increasing rates of chlorsulfuron. This is not adequately reflected in the rapeseed dry weight data. In 1981, neither the one-year nor the two-year carryover resulted in a significant reduction of the rapeseed biomass. Only the 25 g/ha application in 1980 caused some stand thinning and a slight delay in attaining maturity of the rapeseed in 1981.

These field experiments indicate that applications of up to 20 or 25 g/ha of chlorsulfuron in the spring of one year, had no effect on the growth of rapeseed in the following year. Higher rates, up to 150 g/ha, did cause a

Table XIII. Effect of One- and Two-Year Chlorsulfuron Carryover on the Biomass of Rapeseed. St. Albert Site.

Treatment	Rate g/ha	Date	1980-rape Aug.19 Injury score	1980-rape Aug.19 Dry wt. g/m²‡	1981-rape July 3 Injury score	1981-rape July 3 Dry wt. g/m²‡
Weedy control			9	405 a	8	288
Chlorsulfuron	50	June 29,'79	6	270 b	8	311
Chlorsulfuron	100	June 29,'79	6	224 b	9	357
Chlorsulfuron	150	June 29,'79	5	181 b	9	336
Chlorsulfuron	5	July 12,'80			8	306
Chlorsulfuron	10	July 12,'80			8	433
Chlorsulfuron	25	July 12,'80			7	311
LSD 0.05				95		132

‡ Means that are in the same column followed by the same letter are not significantly different at P = 0.05 using Duncan's New Multiple Range Test.

significant, adverse effect one year after application, but they did not influence the growth of rapeseed two years after application.

4.5.2 Corn Root Bioassay

The amount of chlorsulfuron in the soil of some of the plots at the Ellerslie site was determined by means of the corn root bioassay. The relationship between the maximum length of the corn root at the time of harvest and the chlorsulfuron concentration is presented in Figure 13. The sensitivity of this bioassay was between 0 and 6 ppb

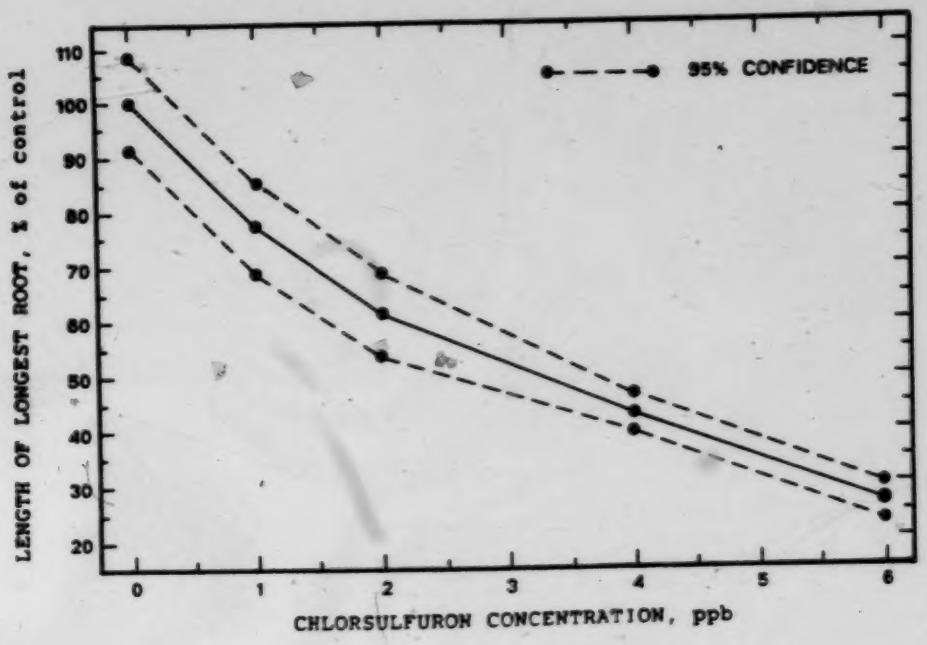

Figure 13. Corn Root Bioassay Standard Curve.

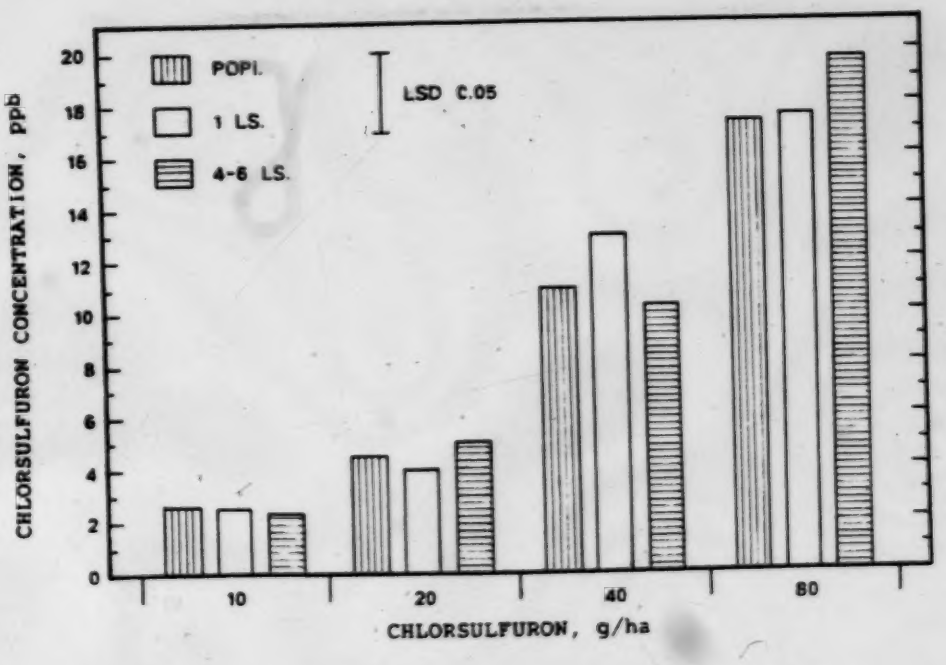

Figure 14. Chlorsulfuron Concentration in the Soil vs Applied Rate.
Application date: June-July 1980. Sampling date: November 3, 1980. Assay date: March 1981.
LSD value on the basis of factorial analysis.

chlorsulfuron in the soil. In order to remain within this sensitive range, the soil samples of those plots that had received 40 or 80 g/ha of chlorsulfuron, were mixed with untreated soil to obtain a dilution ratio of 7:13 and 1:3, respectively.

The time of chlorsulfuron application in the spring had no influence on the concentration of chlorsulfuron in the soil in the fall, regardless of the dosage applied (Figure 14). At all four rates tested there was no significant difference between the POPI treatment and the applications at the 1-leaf stage or at the 4 to 6-leaf stage. The chlorsulfuron concentration in the samples of the plots that had received either 10 or 20 g/ha of chlorsulfuron, were not significantly different from each other. The 40 and 80 g/ha rates did result in chlorsulfuron concentrations that were significantly different from each other and from the two lower rates.

4.5.3 Discussion

The results of the bioassay must be interpreted cautiously (27). The chlorsulfuron concentrations obtained by means of this procedure are an indication of the quantity of phytotoxic residue (carryover) in the soil (Appendix, Table A2). A significant amount of herbicide could be adsorbed to the soil, and consequently unavailable to the roots of the corn seedling (85). If this is assumed to be the case, the assay has two related problems. (i) Since the

standard curve was determined by mixing a solution of chlorsulfuron with ground, air-dried, control soil just prior to planting the pre-germinated corn seed, an equilibrium between the plant-available and the adsorbed chlorsulfuron might not have been reached within the 9-day growing period required by this assay. Consequently, when this standard curve was used to convert the root growth data, obtained by assaying sample soil in which the equilibrium has had several months to become established, into concentrations of plant-available chlorsulfuron, incorrect values might have been obtained. (ii) Mixing control soil with sample soil to adjust the concentration of plant-available chlorsulfuron to be within the sensitive range of this bioassay, might have had an effect beyond the desired dilution. If the equilibrium between the adsorbed and the plant-available chlorsulfuron did not become established rapidly, the final results, based on the multiplication of the chlorsulfuron concentration in the mixture by the dilution factor, could be erroneous.

4.6 Effect of Chlorsulfuron and Hydroxyurea on the Root Growth of Corn Seedlings

When corn seedlings were grown in a 2 ppb chlorsulfuron solution, the root growth rate, expressed as mm/12 hr, was significantly reduced compared to the control root growth rate, beginning at 36 hr after the start of the experiment (Figures 15 and 16). An increase in chlorsulfuron concentration from 2 to 5 ppb had no significant effect (Figure 15 and 17). In a 10 ppb chlorsulfuron solution, the reduction in root growth rate compared to the control became significant at 24 instead of at 36 hr (Figures 15 and 18).

The addition of 10^{-3} M hydroxyurea to the 2 ppb chlorsulfuron solution significantly decreased the growth of the corn roots, beginning at 24 hr (Figure 16). Adding 10^{-3} M hydroxyurea to the 5 and 10 ppb chlorsulfuron solutions had no significant effect on the root growth rate of corn seedlings (Figures 17 and 18). The effect of 10^{-3} M hydroxyurea solution on corn root growth in the absence of chlorsulfuron is shown in Figure 15.

On the basis of what is known about the mode of action of hydroxyurea, in terms of its specificity and the speed with which it acts (Section 2.4.3), it can be assumed that the reduction in the growth rate of corn roots in 10^{-3} M hydroxyurea solution is mainly due to an inhibition of cell division. The observed growth, i.e., increase in root length, must be attributed primarily to the elongation of existing cells. The failure of 2, 5, or 10 ppb chlorsulfuron to reduce this root growth rate of corn seedlings any

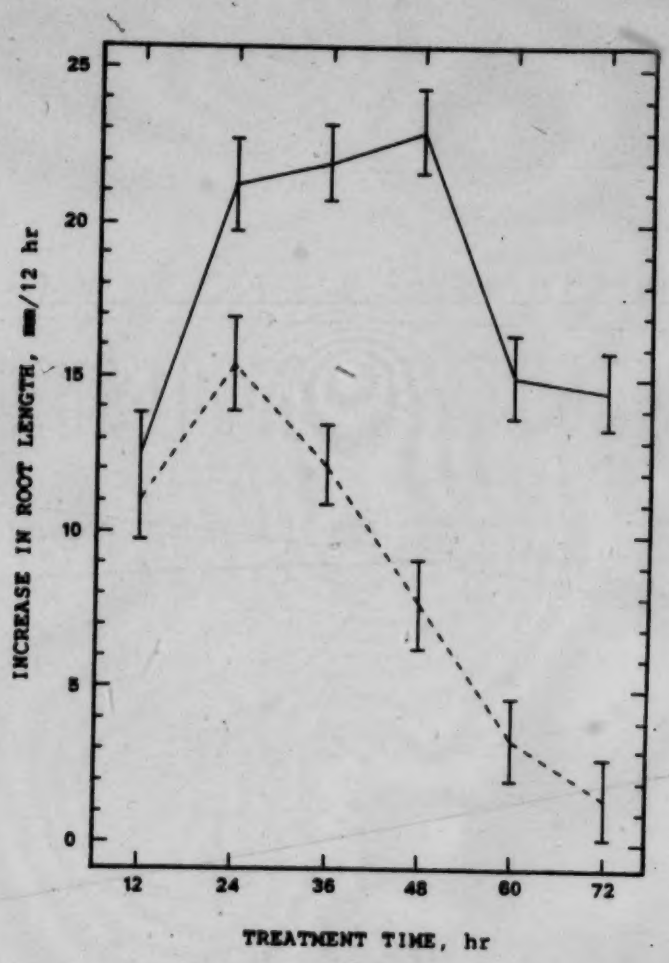

Figure 15. Influence of Hydroxyurea on the Root Growth Rate of Corn Seedlings.
(——) 0 M, (----) 10^{-2} M.

further, suggests that chlorsulfuron does not inhibit cell elongation. In a similar experiment, Hoppe (52) observed that the addition of diclofop methyl, a herbicide known to have an inhibitory action on cell elongation, to 10^{-2} M hydroxyurea, did reduce the root growth rate of corn seedlings beyond the reduction due to the hydroxyurea.

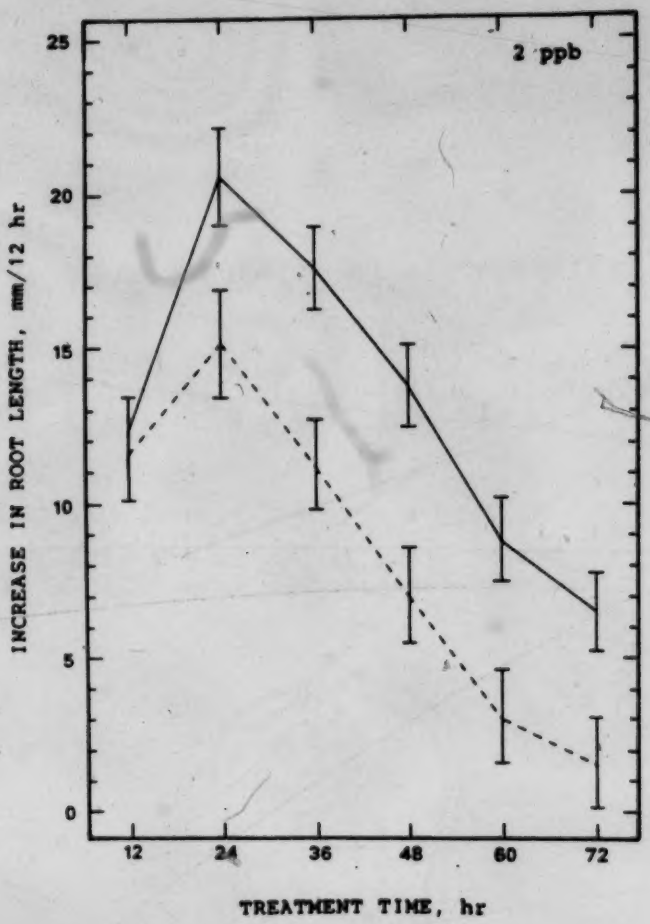

Figure 16. Influence of 2 ppb Chlorsulfuron, with (----) or without (——) 10^{-2} M Hydroxyurea, on the Root Growth Rate of Corn Seedlings.

The failure of 10^{-2} M hydroxyurea to reduce the root growth rate of corn seedlings beyond the reduction induced by 5 and 10 ppb chlorsulfuron, appears to be further evidence that chlorsulfuron inhibits cell division. The ability of 10^{-2} M hydroxyurea to cause a significant effect at 2 ppb chlorsulfuron could reflect the influence of

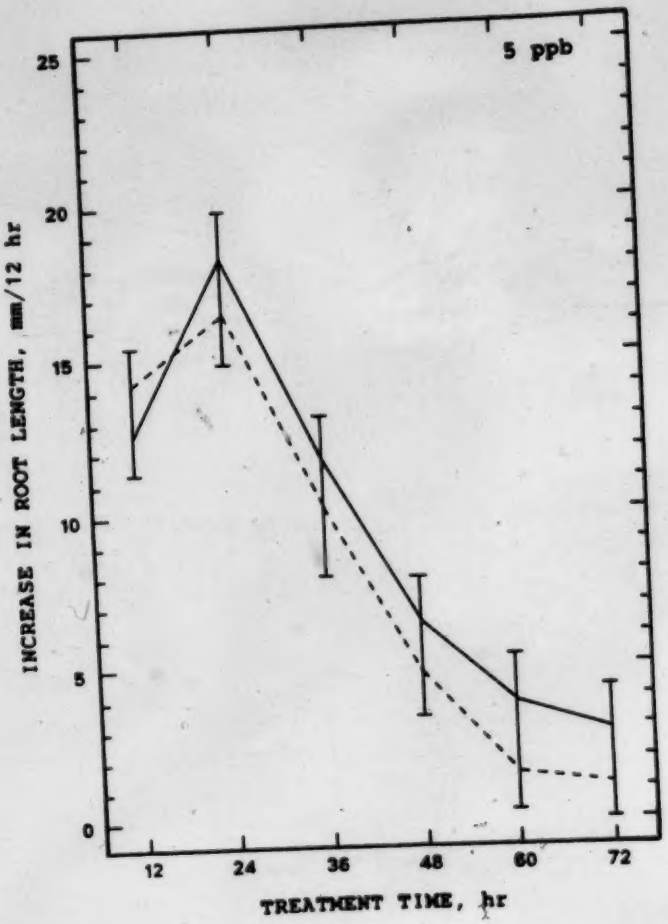

Figure 17. Influence of 5 ppb Chlorsulfuron, with (----) or without (——) 10^{-2} M Hydroxyurea, on the Root Growth Rate of Corn Seedlings.

chlorsulfuron concentration upon the magnitude of its inhibitory action. The basis of the corn root bioassay provides evidence for this also.

The influence of chlorsulfuron, with or without hydroxyurea, on the shoot growth of the corn seedlings was not quantified. Visual observations indicated only minimal

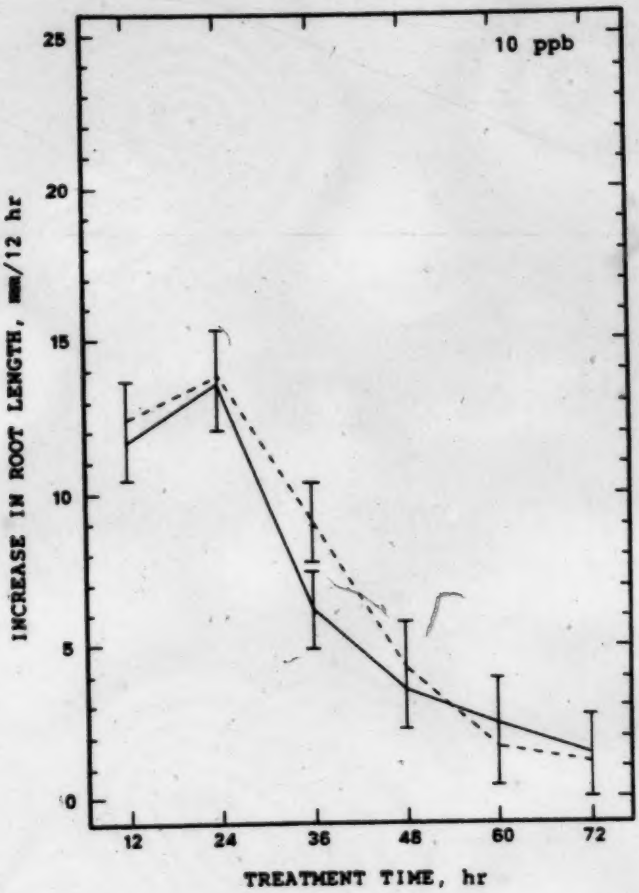

Figure 18. Influence of 10 ppb Chlorsulfuron, with (----) or without (——) 10^{-3} M Hydroxyurea, on the Root Growth Rate of Corn Seedlings.

inhibition of shoot growth.

4.7 Absorption, Translocation, and Metabolism of Chlorsulfuron by Tartary Buckwheat

4.7.1 Absorption and Translocation of ^{14}C-Chlorsulfuron

When Tartary buckwheat plants were harvested 120 hr after a single-droplet application of 1577 Bq of ^{14}C-chlorsulfuron, most of the ^{14}C-activity was recovered from the treated leaf (Table XLV). Of the total amount applied, 42% was recovered in the leaf wipes, and 37% from the tissue of the treated leaf. Approximately 1% was recovered from the stem section above the cotyledonary node and from the third leaf. The remaining parts each contained less than 1% of the applied amount. The total amount of ^{14}C-activity recovered 120 hr after treatment was 81.6% of the applied activity.

When a known amount of ^{14}C-chlorsulfuron was added to non-treated, freeze-dried Tartary buckwheat plant material at the start of the extraction procedure, the ^{14}C-activity in the final extract was 84.5 ± 2.6% (s-) of the applied activity (Appendix, Table A3). Losses due to the transfer of extracts during the procedure accounted for 1.4%, while only 0.1% remained in the plant tissue residue or in the filter paper. The total amount of ^{14}C-activity accounted for was 86.0 ± 2.7% (s-) of the applied activity.

The low recoveries encountered in these ^{14}C-chlorsulfuron experiments are difficult to explain. Even though one of the products in the metabolism of chlorsulfuron is CO_2, it is not expected that the phenyl

Table XIV. ^{14}C-Activity Recovered from Tartary Buckwheat Plants 120 hr after Treatment.

No.	Plant part‡	Recovered DPM % of applied†	
1.	Roots	0.2	(0.08)
2.	Stem below cotyledonary node	0.2	(0.03)
3.	Cotyledonary node	0.2	(0.02)
4.	Cotyledons	0.1	(0.01)
5.	Stem above cotyledonary node	0.9	(0.33)
6.	First true leaf	0.2	(0.05)
7.	Treated leaf (excl. droplet site and leaf wipes)	15.8	(3.94)
8.	Droplet site	20.9	(7.73)
9.	First leaf wipe	40.3	(7.53)
10.	Second leaf wipe	1.5	(0.24)
11.	Third leaf	1.0	(0.22)
12.	Fourth leaf	0.4	(0.04)
	Total recovered	81.6	

† Mean and the standard error of the mean.
‡ Replicate 1 of parts 1 - 6, 11, and 12 was extracted; replicates 2, 3, and 4 were oxidized. All replicates of parts 7 and 8 were extracted.

ring, which contains the ^{14}C-labelled carbon, has been metabolized to $^{14}CO_2$ during these experiments. The CO_2 that is produced in the initial steps of chlorsulfuron metabolism, presumably derives from the carbonyl group of the molecule (Figure 1) (31). The low vapor pressure of chlorsulfuron, 4.6×10^{-9} mm Hg at 25°C, does not support the hypothesis that ^{14}C-activity has been lost due to volatilization. Pipetting errors and errors in liquid scintillation counting must also be disclaimed. Losses due to adsorption of chlorsulfuron to the surface of glass, porcelain, or stainless steel have been found to be

insignificant (31).

If the roots of the Tartary buckwheat plants would exude chlorsulfuron, some ^{14}C-activity could possibly be recovered from the cups containing the vermiculite or the nutrient solution. No ^{14}C-activity was found in the latter, while the vermiculite in the former was not analyzed. Since translocation of chlorsulfuron was found to be minimal, root exudation of chlorsulfuron also is not expected to be a major source of loss.

At 1, 24, 120, and 240 hr after treatment, the total amounts of ^{14}C-activity, expressed as percentages of the applied quantity, recovered from the treated leaves[1], were similar (Figure 19). At each of the four harvest times, approximately 79% of the applied activity was recovered from the treated leaf. During this time period, 1 to 120 hr, the chlorsulfuron was absorbed by the leaf tissue and translocated away from the site of application, as indicated by the significant increase in the amount of ^{14}C-activity in the treated leaf, excluding the droplet application site and the leaf wipes. The increase in the recovery of ^{14}C-activity from the tissue of the treated leaf (treated leaf including droplet site but excluding leaf wipes), can be attributed to absorption of ^{14}C-chlorsulfuron by the leaf tissue; witness the concurrent decline in the amount recovered from the first leaf wipe. The amount recovered from the second leaf

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[1] The treated leaf includes the droplet application site and the two leaf wipes.

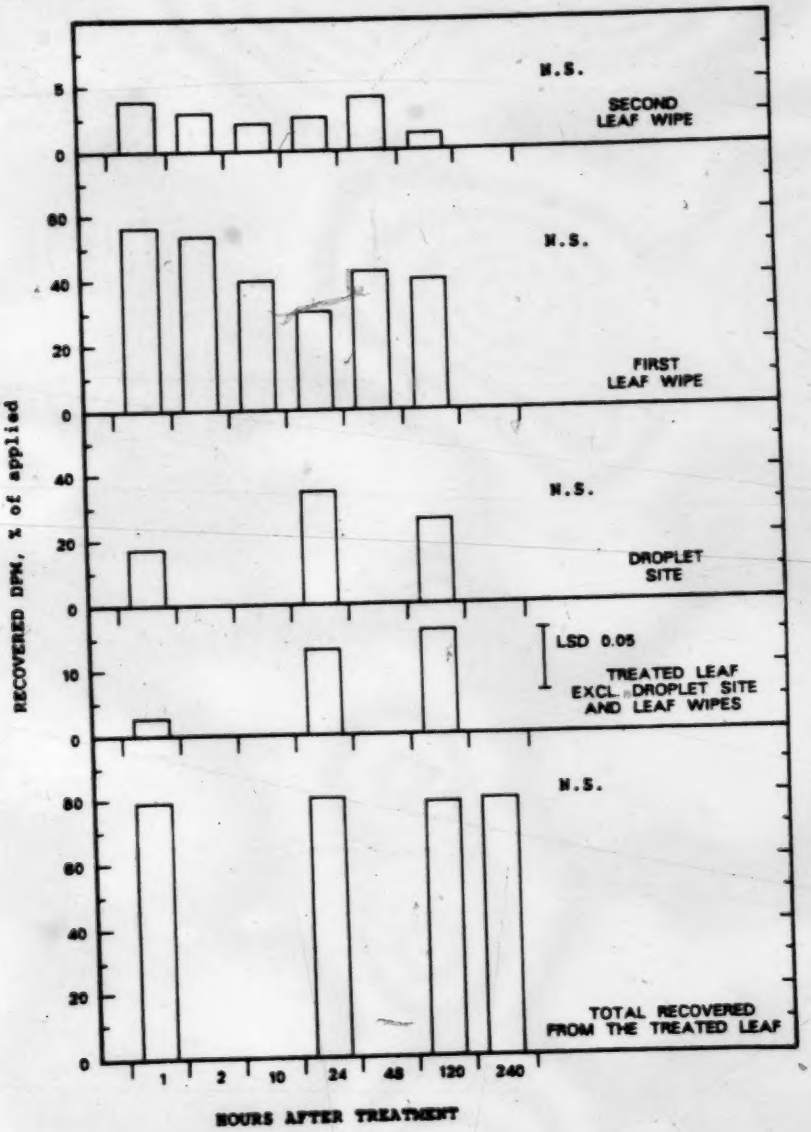

Figure 19. ^{14}C-Activity Recovered from the Treated Leaf of Tartary Buckwheat Plants at Seven Different Times after the Application of ^{14}C-Chlorsulfuron.
N.S. indicates F-value was not significant at P = 0.05.

wipe was not dependent upon the time after treatment.

Because the performance of the extraction procedure was monitored routinely, it was observed that the extractant was not able to extract all the ^{14}C-activity from the samples. Large quantities of ^{14}C-activity were detected when the filter paper discs, together with the retained plant residue, were oxidized (Appendix, Table A4). When ^{14}C-chlorsulfuron was added to non-treated, freeze-dried Tartary buckwheat tissue at the start of the extraction procedure, the amount of ^{14}C-activity retained by the filter paper discs and the ground tissue was insignificant (Appendix, Table A3). Rather than being adsorbed by the filter paper, ^{14}C-chlorsulfuron, or a ^{14}C-metabolite, appeared to be adsorbed by the plant tissue of the samples.

The failure of the methanol-acetone extractant to extract all the ^{14}C-activity from the plant tissue can be seen in the light of two related factors: (i) the amount of time during which the tissue was exposed to the extractant, and (ii) the degree of adsorption of chlorsulfuron or its metabolite(s) to the plant tissue. Because hydrolysis of chlorsulfuron in protic solvents was anticipated, the time during which the tissue was exposed to the methanol-acetone extractant was kept to a minimum. The average extraction period was 2 to 4 min. No data regarding the degree of adsorption can be submitted, since neither the effect of longer extraction periods nor the performance of different extractants was investigated.

4.7.2 Metabolism of ^{14}C-Chlorsulfuron

When several chromatograms of non-hydrolyzed and partially-hydrolyzed ^{14}C-chlorsulfuron were compared, the Rf value of the ^{14}C-chlorsulfuron molecule was observed to be between 0.81 and 0.83. The Rf value of the ^{14}C-hydrolysis product ranged between 0.53 and 0.61. Because the phenyl ring of the chlorsulfuron molecule contains the ^{14}C-label, this breakdown product presumably is the 2-chlorobenzene-sulfonamide part. This hydrolysis product was not further identified. An attempt was made to locate the other hydrolysis product, the 2-amino-4-methoxy-6-methyl-1,3,5-triazine part, on the chromatograms. Although this structure does absorb ultraviolet light, viewing the chromatograms under this light did not reveal its location.

Table XV presents the results obtained when aliquots of the extracts of the treated leaf at 120 and 240 hr after treatment were chromatographed and the amounts of radioactivity in the two bands, each containing one possible radioactive spot, were determined. The radiochemical purity of the applied droplet was 95.9%.

Even though it appears that very little ^{14}C-chlorsulfuron metabolism has occurred, in the light of the previously reported failure to extract all the ^{14}C-activity from the plant tissue, these results must be interpreted with caution. Because it is not known if the non-extracted ^{14}C-activity (Appendix, Table A4) is ^{14}C-chlorsulfuron or a ^{14}C-metabolite, no conclusive

Table XV. **Results of Reverse Phase Thin Layer Chromatography of the Applied ^{14}C-Chlorsulfuron Solution and of the Extracts of the Treated Leaf of Tartary Buckwheat Plants 120 and 240 hr after Treatment.**

Description‡	^{14}C-activity recovered % of amount spotted on RPTLC plate†		
	Rf 0.44-0.77	Rf 0.77-1.00	Total
Plant-applied droplet	1.2 (0.1)	95.9 (0.5)	97.1 (0.5)
120 HAT: part 7	7.7 (1.1)	86.1 (1.4)	93.8 (0.4)
part 8	5.4 (2.2)	90.6 (2.9)	96.0 (1.0)
240 HAT: part 7 + 8	3.5 (0.3)	90.9 (2.2)	94.3 (2.2)

† The amount spotted on the plate was determined by placing a similar amount in a liquid scintillation vial and counting it.
Mean and standard error on the basis of four replicates.

‡ Part 7: treated leaf excluding the droplet application site and the leaf wipes. Part 8: droplet application site.

statement regarding chlorsulfuron metabolism by Tartary buckwheat can be made.

4.7.3 Mutilation Study

The effect of allowing a treated leaf to be connected to the main stem of a Tartary buckwheat plant for different time periods, following a single-droplet application of 7 ug chlorsulfuron, has been recorded in Figure 20 in terms of the shoot dry weight 14 days after treatment. A 2-hr period was sufficient to result in a shoot dry weight significantly below that of control plants. The symptoms induced in the

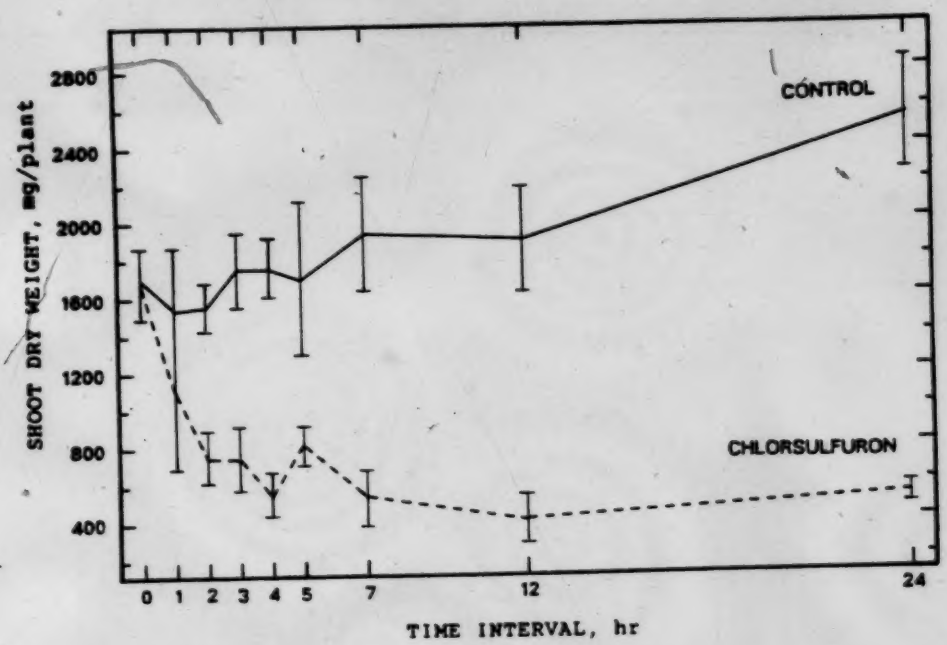

Figure 20. Effect of Removal of the Treated Leaf of Tartary Buckwheat Plants at Different Time Periods Following a Single-Droplet Application of Chlorsulfuron, 7 ug/Plant, 2-Leaf Stage, on the Shoot Dry Weight 14 Days after Treatment.

Tartary buckwheat plants were similar to the ones observed when the treated leaf was not removed after the application of chlorsulfuron. Within 24 hr after treatment, chlorosis of the apex could be observed in all plants. The stem of the chlorsulfuron-treated plants did not elongate, and the plants were considerably slower in their development. Three days after treatment, the treated plants became slightly chlorotic. None of these symptoms was observed in the control plants.

4.7.4 Discussion

When, in the light of the chlorsulfuron-induced symptoms, the mutilation study is related to the ^{14}C-chlorsulfuron experiment, several hypotheses regarding the physiological behaviour of chlorsulfuron in Tartary buckwheat can be advanced. If it is assumed that chlorsulfuron molecules have to be present in the main stem in a certain minimum concentration in order to inhibit growth, sufficient translocation must take place within the 2-hr period following application. Even though, on the basis of the technique used in this study, translocation during this time period was minimal, sufficient translocation appears to have occurred.

If it is assumed that chlorsulfuron molecules do not have to be present in the main stem in order to inhibit growth, chlorsulfuron may exert its effect through another agent, possibly through growth hormones. When, after a period of inhibition, the length of this period being dependent upon the leaf stage at the time of application, the growth of chlorsulfuron-treated Tartary buckwheat plants resumed, the main stem failed to elongate. This failure might be due to a direct effect of chlorsulfuron on the stem elongation process, or an indirect effect by means of growth hormones. The latter has been reported by Kefford (56) for chloroethyltrimethylammonium chloride (chlormequat chloride; CYCOCEL).

5. SUMMARY AND CONCLUSIONS

Preemergence and postplanting-incorporated treatments of up to 80 g/ha of chlorsulfuron did not provide an acceptable level of Tartary buckwheat control in the field. The efficacy of postemergence chlorsulfuron applications for the control of Tartary buckwheat in cereal crops was dependent upon the leaf stage of the Tartary buckwheat at the time of spraying and upon the rate of application. This efficacy was rather inconsistent. In some instances, rates as low as 10 or 40 g/ha were sufficienct to control Tartary buckwheat when applied at the 1- or 4 to 6-leaf stage respectively. At other times, rates up to 60 g/ha provided only limited control, regardless of the leaf stage at the time of application.

In order to explain this inconsistency in chlorsulfuron efficacy the influence of temperature, relative humidity, soil moisture, and light intensity should be determined. Because these factors were not adequately monitored in the micro-environment of the Tartary buckwheat plants in the field, no satisfactory explanation for the observed inconsistency in chlorsulfuron efficacy can be provided.

Recommendations regarding the use of chlorsulfuron for the control of Tartary buckwheat in cereal crops in north-central Alberta must reflect the increase in the rate required to achieve satisfactory control at advanced leaf stages. The lack of uniformity of a natural infestation of Tartary buckwheat in a farmer's field will require an

application rate that is based on the plants with the most advanced leaf stage. An understanding of the influence of environmental factors on chlorsulfuron efficacy could further modify these recommendations.

Chlorsulfuron is an effective herbicide for the control of hempnettle and cleavers, both in cereal crops and on fallow. Rates as low as 5 g/ha gave excellent control of hempnettle plants that had eight true leaves at the time of application, while a minimum of 30 g/ha effectively controlled cleavers that had two whorls of leaves at the time of application.

Controlled environment studies revealed that the susceptibility of Tartary buckwheat plants to chlorsulfuron decreased as the leaf stage at the time of application advanced from one to four, regardless of the chlorsulfuron dosage. This degree of susceptibility was reflected in the length of the period following chlorsulfuron application during which growth was inhibited, ranging from 10 or more days for applications at the 1-leaf stage to 1 - 2 days for applications at the 4-leaf stage. Although plants that resumed growth after the inhibition period developed normal leaves and flowers, the main stem failed to elongate. At the 1-, 2-, and 3-leaf stage, chlorsulfuron caused a shriveling of the main stem of the Tartary buckwheat plants below the cotyledonary node.

In controlled environment experiments a significant effect of the adjuvant Cittowet Plus on the efficacy of

chlorsulfuron on Tartary buckwheat was demonstrated in terms of the shoot dry weight 18 days after treatment. In the field, chlorsulfuron-induced symptoms were more severe in the presence of Cittowet Plus in the spray solution than in its absence. However, no significant effect in terms of shoot dry weight at harvest time, 59 or 72 days after treatment, was observed. Because chlorsulfuron causes only a temporary growth inhibition of Tartary buckwheat plants, the difference in chlorsulfuron-induced symptoms or shoot dry weight reductions in the first few weeks following application due to the adjuvant, diminishes as the plants get older. Hence the failure of Cittowet Plus to reduce the shoot dry weight of Tartary buckwheat plants in the field at harvest time significantly.

In the field, chlorsulfuron at up to 40 g/ha did not significantly reduce the efficacy of the postemergence wild oat herbicides diclofop methyl, flamprop methyl, difenzoquat, and barban when applied as a tank mix. In the controlled environment, chlorsulfuron rates as high as 80 g/ha in tank mixes also had no significant adverse effect on the efficacy of these wild oat herbicides. None of these postemergence wild oat herbicides had a significant effect on chlorsulfuron efficacy. These results suggest that a single application of a tank mix of chlorsulfuron with one of the four wild oat herbicides, in order to control both chlorsulfuron-susceptible broadleaved weeds and wild oats in cereal crops, can be used without a reduction in either

broadleaved weed or wild oat control, compared to the results of two separate applications of these herbicides.

Foliage applications of chlorsulfuron at up to 80 g/ha did not cause any significant injury to wheat or barley under field conditions. In the controlled environment, chlorsulfuron up to 40 g/ha had no visible effect on the growth of wheat or oats. Barley plants suffered a reduction in dry weight. Although higher rates, up to 360 g/ha, reduced the dry weight of all species, mainly due to reductions in internode length, no delay in maturity was observed. At rates required for acceptable control of Tartary buckwheat, hempnettle, and cleavers, postemergence applications of chlorsulfuron did not injure wheat, barley, and oats.

Chlorsulfuron applications of up to 25 g/ha in the spring of one year had no significant effect on the growth of rapeseed in the following year. Higher rates caused a significant reduction in rapeseed biomass, due to a thinning of the stand, and a delay in attaining maturity. A two-year interval between the time of a 50 to 150 g/ha chlorsulfuron application and the seeding of rapeseed proved to be sufficient for the chlorsulfuron residues to dissipate below a level inhibitory to the growth of rapeseed. Pending further research regarding the factors influencing chlorsulfuron degradation in the soil, the use of chlorsulfuron is not recommended if rapeseed is to be grown the following year.

The corn root bioassay proved to be a useful method for determining the chlorsulfuron concentration in soil. This assay was able to detect between 0 and 6 ppb. Although the maximum chlorsulfuron concentration in the soil that did not significantly affect the growth of rapeseed could not be deduced from this study, it appears that 18 ppb in the soil, in the fall, is sufficient to cause a significant inhibition of rapeseed growth the following year.

The root growth rate of corn seedlings was significantly reduced by 2, 5, and 10 ppb chlorsulfuron solutions. On the basis of the interaction of these chlorsulfuron concentrations with 10^{-2} M hydroxyurea, it is postulated that inhibition of cell division is the predominant effect of chlorsulfuron in corn roots.

The translocation of chlorsulfuron in Tartary buckwheat is minimal. At 1, 24, 120, and 240 hr after a single-droplet application of ^{14}C-chlorsulfuron, approximately 79% of the applied activity was recovered from the treated leaf. The remaining plant parts contained 2.6%. The total recovery was 81.6% of the applied activity. No explanation for this low recovery can be provided.

Chlorsulfuron is not rapidly absorbed by the leaf tissue of Tartary buckwheat. At 120 hr after treatment, 37% of the applied ^{14}C-activity was recovered from the tissue of the treated leaf; 42% was recovered in the leaf wipes.

Chromatographic analysis of the plant extracts revealed that very limited metabolism of the ^{14}C-chlorsulfuron had

occurred 120 and 240 hr after treatment. Because the extractant failed to extract all the ^{14}C-activity from the plant tissue, no definitive statement regarding the rate of chlorsulfuron metabolism can be made.

A leaf of a Tartary buckwheat plant, treated with a single droplet of chlorsulfuron solution, had to remain connected to the untreated remainder of the plant for only 2 hours in order to induce typical chlorsulfuron symptoms in that plant. It is suggested that either the limited amount of chlorsulfuron that is translocated in the 2-hour period is sufficient to induce these symptoms, or chlorsulfuron acts through another, readily translocated agent.

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APPENDIX

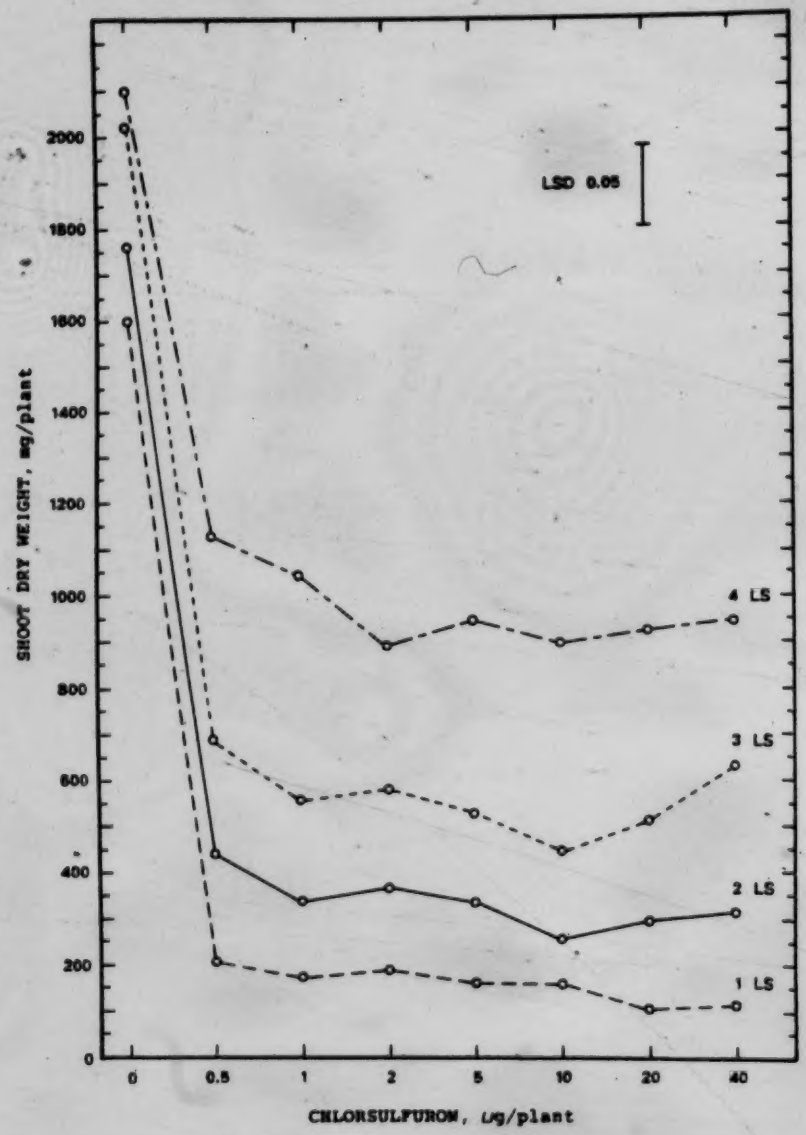

Figure A1. Effect of Increasing Dosages of Chlorsulfuron, in Single-Droplet Application at Four Different Leaf Stages, on the Shoot Dry Weight of Tartary Buckwheat Plants, Harvested 14 Days after Treatment.
Chlorsulfuron dosages are plotted on logarithmic scale.
LSD value is between dosages of chlorsulfuron at the same leaf stage.

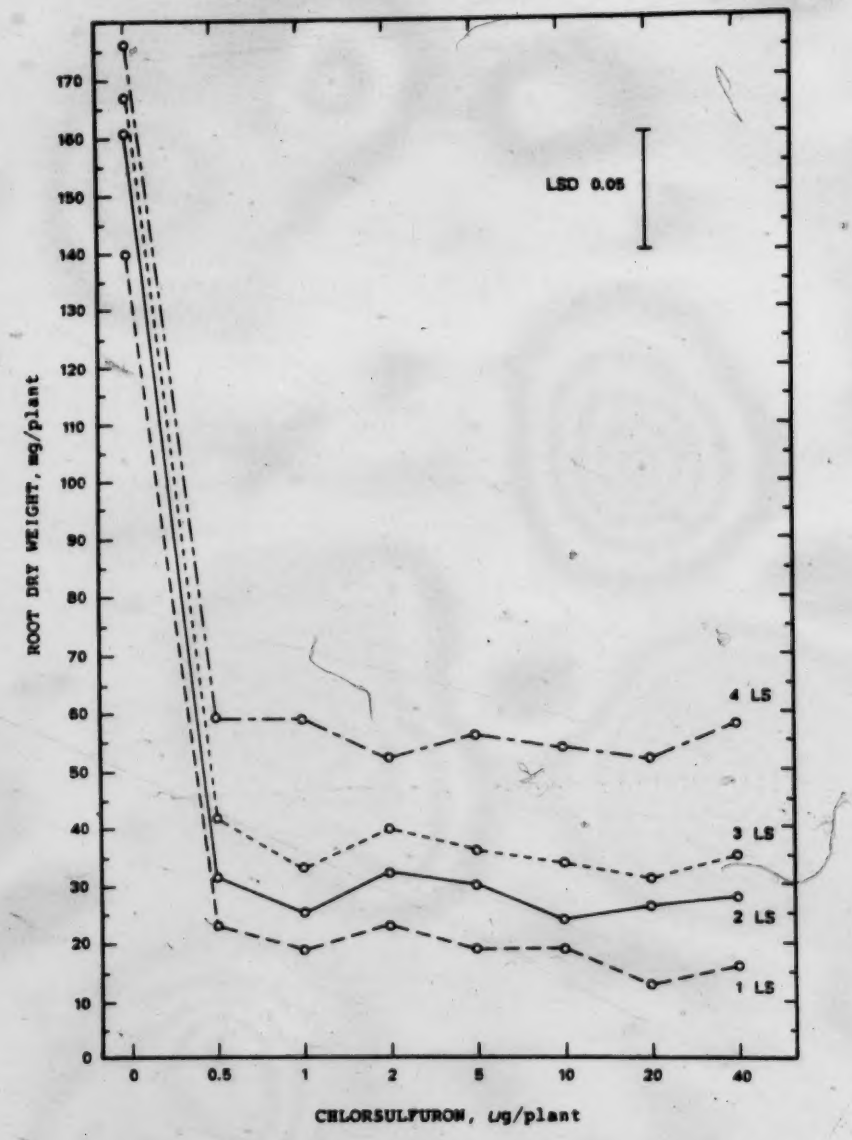

Figure A2. Effect of Increasing Dosages of Chlorsulfuron, in Single-Droplet Application at Four Different Leaf Stages, on the Root Dry Weight of Tartary Buckwheat Plants, Harvested 14 Days after Treatment.
Chlorsulfuron dosages are plotted on logarithmic scale.
LSD value is between dosages of chlorsulfuron at the same leaf stage.

Table A1. **Weather Data of the Ellerslie Site at the Time of Chlorsulfuron Application. 1980 and 1981 Tartary Buckwheat Control Experiment.**

Chlorsulfuron application			Weather data‡			
Date	Time hrs	LS†	Max. temp. °C	Total rainfall mm	RH %	Total sunshine hrs
June 19, 1980			20.5	0.2	84	
June 20, 1980	0700	1	25.0	-	67	
July 2, 1980			26.0	-	52	
July 3, 1980	0700	2-3	24.5	22.8	63	
July 7, 1980	2100	4-6	23.5	-	65	
July 8, 1980			24.5	-	60	
June 2, 1981	2030	2	21.1	-	68	14.4
June 2, 1981			24.4	10.6	94	8.0
June 15, 1981	2030	5	21.8	0.2	57	11.9
June 16, 1981			20.2	1.8	77	3.3

† Leaf stage refers to the number of true leaves of the Tartary buckwheat plants at the time of chlorsulfuron application.

‡ The weather data were recorded each day at 0900 hrs. Source: Ellerslie agrometereological station, Department of Geography, University of Alberta, Edmonton.

Table A2. Chlorsulfuron Concentration Data Obtained by the Corn Root Bioassay.

Rate g/ha	Time†	Spray date 1980	Chlorsulfuron concentration, ppb ¥				
			Rep. I ‡	Rep. II ‡	Rep. III ‡	Rep. IV ‡	Mean§
10	POPI	June 12	4.4(2.9- 5.6)	0.7(0 - 1.6)	2.5(1.4- 3.6)	2.8(1.3- 4.4)	2.6(0.8)
20	POPI	June 12	5.2(4.2- 6.0)	2.4(1.4- 3.4)	4.7(3.2- 6.0)	5.9(5.0- 6.8)	4.6(0.8)
40	POPI	June 12	14.0(12.0-15.7)	12.6(8.3-16.3)	8.6(5.4-12.3)	8.6(5.4-12.0)	11.0(1.4)
80	POPI	June 12	18.8(14.8-22.8)	9.6(5.6-14.4)	20.8(18.4-23.6)	20.0(16.0-23.2)	17.3(2.6)
10	1 LS	June 20	2.7(1.8- 3.9)	1.2(0.1- 2.0)	2.8(1.4- 4.0)	3.5(2.8- 4.8)	2.6(0.5)
20	1 LS	June 20	3.9(2.3- 5.1)	3.4(1.8- 4.7)	3.8(2.6- 4.7)	4.7(3.8- 5.7)	4.0(0.3)
40	1 LS	June 20	16.6(14.3-18.9)	10.3(6.6-12.9)	11.4(7.4-14.8)	13.4(7.4-14.8)	12.9(1.4)
80	1 LS	June 20	22.8(20.0-25.6)	13.6(8.0-17.6)	16.4(12.4-19.6)	17.2(12.0-21.6)	17.5(1.9)
10	4-6 LS	July 7	3.2(2.0- 4.1)	1.0(0.2- 2.1)	2.9(1.3- 4.5)	2.1(0.9- 3.7)	2.3(0.5)
20	4-6 LS	July 7	5.5(4.3- 6.7)	4.1(2.6- 5.3)	5.4(4.8- 6.0)	5.0(4.2- 5.7)	5.0(0.3)
40	4-6 LS	July 7	10.6(5.7-14.8)	6.3(3.4-10.6)	12.3(9.4-15.1)	11.4(7.4-14.9)	10.2(1.3)
80	4-6 LS	July 7	22.0(16.8-27.2)	14.4(9.6-17.2)	22.8(19.6-26.0)	19.6(15.6-23.6)	19.7(1.9)
LSD 0.05							1.5

† Leaf stage refers to the number of true leaves of the Tartary buckwheat plants at the time of spraying.
§ Mean and standard error on the basis of four field replicates.
‡ Mean and the range on the basis of six bioassay replicates.
¥ Sampling date: Nov. 1980. Assay date: March 1981.

Table A3. Recovery of ^{14}C-Activity from Tartary Buckwheat after Adding ^{14}C-Chlorsulfuron to the Freeze-Dried Plant Material just prior to Extraction.

Description	DPM recovered % of applied†
Polytron grinder	0.74 (0.11)
Glassware: centrifuge tube	0.07 (0.02)
boiling flask	0.60 (0.07)
Filter paper and plant residue	0.11 (0.01)
Plant extract	84.49 (2.59)
Total recovered	86.01 (2.69)

† Mean and standard error on the basis of three replicates.

Table A4. Amount of ^{14}C-Activity Recovered by Extraction and by Oxidizing the Filter Paper with Retained Residue of Different Parts at Four Different Times Following Treatment of Tartary Buckwheat Plants with ^{14}C-Chlorsulfuron.

Treatment	Part†	^{14}C-activity recovered‡		
		Extract	Residue and filter paper	
		DPM	DPM	%
1	7	924 (603)	1602 (615)	63.4
	8	14313 (3783)	422 (230)	2.9
	11	215 (51)	30 (9)	12.2
24	7	7475 (330)	4394 (306)	37.0
	8	31645 (3038)	381 (93)	1.2
	11	270 (97)	628 (281)	1.4
	12	53 (11)	196 (91)	78.7
120	7	7583 (3144)	8322 (2313)	52.3
	8	14125 (6870)	4021 (3092)	22.2
	11	256 (71)	941 (123)	78.6
	12	60 (13)	295 (59)	83.1
240	(7,8,9,10)	47063 (3896)	26141 (2474)	35.7
	11	860 (119)	1250 (312)	59.2
	12	298 (110)	480 (133)	61.7

† Part 7: treated leaf excluding droplet application site. Part 8: droplet application site. Part 9: first leaf wipe. Part 10: second leaf wipe. Part 11: third leaf. Part 12: fourth leaf.

‡ Mean and standard error on the basis of four replicates.